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THE RELATIONSHIP OF TEMPERATURE AND STAGE OF GROWTH TO THE CROWN RUST REACTION OF CERTAIN VARIETIES OF OATS[1]

M. D. Simons[2]

SUMMARY

Eleven varieties of oats at 4 stages of growth (seedling, juvenile, boot and anthesis) were inoculated simultaneously with race 205 of crown rust. One lot was incubated at about 15° C. and another at about 25° C. With 1 exception, varieties differing in reaction were more resistant at the low temperature and in the later stages of growth. Some varieties, such as Markton, reacted similarly at both temperatures and at all stages of growth. In susceptible varieties older leaves were more resistant than younger leaves on the same plant. Mo. 0-205, completely susceptible in the seedling stage, showed a very high degree of mature plant resistance at both temperatures. At 15° C., Appler, Marion, and Cherokee, also susceptible in the seedling stage, exhibited moderate mature plant resistance. At 25° C. these varieties were susceptible at all stages of growth. Santa Fe, Bondvic, Clintafe and Clintland were uniformly, highly resistant at both temperatures at all stages of growth. Landhafer was highly resistant at 15° C., but at 25° C. this resistance broke down sufficiently at all stages to differentiate it clearly from the other highly resistant varieties.

Telial development was somewhat erratic, but was heavier at 25° C. than at 15° C. on all but the most susceptible varieties. Stages of growth had little or no effect on telial development at 15° C., but at 25° C. telia were produced more abundantly on leaves of plants in the juvenile stage than on plants in the boot or at anthesis.

It is generally recognized that certain varieties of oats respond differently to races of crown rust under different temperatures and at different stages of growth. This investigation was undertaken to obtain information regarding the reactions of 11 selected varieties of oats to a pure race of crown rust at low and high temperatures, and at 4 different stages of growth.

Peturson (6) and Murphy (3) have clearly demonstrated the importance of temperature in influencing the reaction of certain varieties of oats in the seedling stage to some races of crown rust. They also showed that reactions induced by certain other races were little affected by temperature. According to Parker (5), Durrell and Parker (1), and Newton, Johnson, and Peturson (4) there was little evidence of significant variation between seedling and adult plant reactions to crown rust. Murphy (3), however, found certain varieties susceptible as seedlings that showed considerable resistance as older plants in the field. Peturson (7) confirmed this, and further showed that adult resistance to 1 race of crown rust did not necessarily imply resistance to other races.

Contrary to the observations of other investigators, Gassner (2) stated that oat plants naturally infected in the field became more susceptible as they grew older.

Parker (5) and Peturson (6) studied the effect of different environmental conditions on the formation of telia of crown rust, and concluded that telia formed more readily at moderately warm temperatures than at cool temperatures. Telia also formed more readily on plants of resistant varieties than on susceptible varieties.

MATERIALS AND METHODS.—Eleven varieties of oats (Table 1) were used. These were planted in 6 replications and at suitable intervals to provide plants for simultaneous inoculation at 4 stages of growth, seedling, juvenile, boot, and anthesis.[3] A pure culture of race 205 (an atypical biotype of old race 45) of crown rust (*Puccinia coronata* Corda var. *avenae* Fraser and Ledingham) was used. Inoculation was accomplished by spraying the plants with a weak detergent, and dusting the moistened leaves with a urediospore—talc mixture.

After removal from the humidity chamber, 3 replications of the plants were placed in a greenhouse room at 15° C., and the other 3 at 25° C. Plants in the warm room were examined for reaction to rust 10 days after inoculation, and again 6 days later. Plants in the cool room were examined 14 days after inoculation, and again 6 days later. Separate readings were made of the upper and lower leaves of the juvenile plants, and of the upper, mid, and lower leaves of plants in the boot and at anthesis. Uredial development was recorded as (I) no macroscopic evidence of infection, (0) necrosis or chlorosis, but no uredia, (1) necrotic or chlorotic areas a few of which contain small uredia, (2) necrotic or chlorotic areas most of which contain small to mid-sized uredia, (3) chlorotic areas surrounding abundant, mid-sized uredia, no necrosis, (4) no necrosis or chlorosis, uredia large and abundant. Formation of telia was recorded as none (0), light (1), moderate (2), or heavy (3). All data used represented the average of 3 replications.

DEVELOPMENT OF UREDIA.—In general, the development of the uredial stage of the disease at 15° C. was

[1] Accepted for publication January 15, 1954.

[2] Plant Pathologist, Division of Cereal Crops and Diseases, U. S. Department of Agriculture; and Department of Botany and Plant Pathology, Iowa Agricultural Experiment Station, Ames, Iowa. Journal Paper No. J-2424 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 1176.

[3] Santa Fe was tested at only 3 stages (seeding, juvenile, and boot) as the oldest planting had not headed at the time the plants were inoculated.

TABLE 1.—*Average uredial reactions[a] to crown rust race 205 of 11 varieties of oats inoculated simultaneously at 4 stages of growth and then held at 2 temperatures*

Variety	15° C.				25° C.			
	Seedling	Juvenile	Boot	Anthesis	Seedling	Juvenile	Boot	Anthesis
Victoria	0	0	0	0	I	I	0	0
Appler	3.8	2.9	1.1	2.0	4.0	3.0	3.2	3.2
Landhafer	0	0	0	0	1.0	0.9	1.4	1.0
Santa Fe	0	0	0	–	0	0	0	–
Bondvic	0	0	0	0	0	0	0	0
Marion	4.0	3.2	2.5	2.7	4.0	3.2	3.4	3.0
Cherokee	4.0	3.2	2.8	2.6	4.0	3.5	3.4	3.3
Mo. 0-205	3.0	0.9	0	0	4.0	3.1	1.7	1.0
Clintafe	0	0	0.2	0	0	0	0	0.8
Clintland	0	0	0	0	0	0	0	0
Markton	4.0	3.5	3.7	3.5	4.0	3.9	3.7	3.7

[a] "I" indicates no macroscopic evidence of infection; "O", necrosis or chlorosis, but no uredia; "1", necrotic or chlorotic areas, a few of which contain small uredia; "2", necrotic or chlorotic areas, most of which contain small to mid-sized uredia; "3", chlorotic areas surrounding abundant, mid-sized uredia, no necrosis; "4", no necrosis or chlorosis, uredia large and abundant.

about 4 or 5 days behind that at 25° C. In most of the varieties the ultimate reaction differed little at the 2 temperatures (Table 1). Several, however, showed relatively small but consistent differences, and a few showed pronounced differences. These were almost invariably in the direction of greater susceptibility at high temperatures. Landhafer, for example, consistently showed a uniform "0" type reaction in all stages of growth at 15° C. whereas a mesothetic reaction developed at 25° C. Single leaves in some cases exhibited reactions ranging from "0" to "3". The more resistant types were in the majority so that the average reaction was about "1+". This high temperature reaction did not vary appreciably with the different stages of growth. In marked contrast to Landhafer, the varieties Santa Fe, Clintafe, Clintland, and Bondvic exhibited rather uniform "0" type reactions not only at 15° C. but also at the higher temperature. Thus while Landhafer was pathologically indistinguishable from these last named varieties at 15° C. it was readily differentiated at 25° C.

Varieties commonly believed to possess some degree of resistance or tolerance such as Appler, Marion, Cherokee, and Mo. 0-205 appeared on the average to be somewhat more susceptible at high than at low temperatures. The differences, in all cases, were small.

With the possible exception of Victoria, the highly resistant varieties studied (Landhafer, Santa Fe, Bondvic, Clintafe, and Clintland) showed consistent reactions at all stages of growth for any given temperature. Clintland was typical of this group, exhibiting clear-cut, uniform "0" types at all stages and at both temperatures. While Landhafer showed some susceptibility at 25° C. it was consistent among the different stages of growth at a given temperature.

With the exception of the highly susceptible variety Markton, the remaining varieties (Appler, Marion, Cherokee, and Mo. 0-205) all showed some variability in reaction at the different stages of growth. In all cases, resistance increased as the plants grew older. This tendency, in general, was more pronounced at low than at high temperatures.

In Cherokee and Marion, these differences were small, ranging, at 15° C., from "4" types in the seedling stage to "3" at anthesis. At the same temperature Appler was also completely susceptible in the seedling stage, but was only moderately susceptible in the juvenile stage and was moderately resistant in the boot and at anthesis. At 25° C., these 3 varieties differed little among themselves and ranged from completely susceptible in the seedling stage to moderately susceptible at anthesis.

Mo. 0-205 provided the greatest variety of reaction types at different stages of growth. At 15° C. this variety was almost completely susceptible in the seedling stage. In the juvenile stage the reaction had dropped to "1", whereas in the boot and at anthesis it was highly resistant, showing a uniform "0" type reaction. Differences at 25° C. were almost as striking. Seedling and juvenile plants were susceptible; plants in the boot were moderately resistant, and plants at anthesis were highly resistant.

Victoria provided an exception to the usual reaction of greater resistance at lower temperatures and at more advanced stages of growth. At 25° C. Victoria was immune at seedling and juvenile stages of growth and produced a "0" type reaction in boot and anthesis stages. The "0" type reaction developed at all stages at 15° C. Thus, while immune and "0" type reactions are both classed as highly resistant, the difference between them is so clear-cut that Victoria can be considered as most resistant in the early stages of growth and at high temperatures. This unique behavior was confirmed for the seedling stage in a second experiment.

Separate readings were made on leaves of different ages on individual plants, making it possible to determine the influence of the age of the leaf on rust reaction. Appler, Mo. 0-205, Marion and Cherokee, varieties whose reactions were influenced by stage of growth, all showed somewhat greater infection on the

TABLE 2.—*Average telial reactions[a] of 11 varieties of oats in 3 stages of growth at 2 temperatures to crown rust race 205*

Variety	15°C. Juvenile	15°C. Boot	15°C. Anthesis	25°C. Juvenile	25°C. Boot	25°C. Anthesis
Victoria	0	0	0	0	0	0
Appler	0.6	0.8	1.0	2.0	1.0	1.0
Landhafer	0	0	0.3	3.0	2.0	1.8
Santa Fe	0	0	–	0	0	–
Bondvic	0	0	0	1.5	0.3	0.5
Marion	1.0	1.7	2.0	2.0	1.7	2.0
Cherokee	2.5	1.7	3.0	2.0	1.7	1.7
Mo. 0-205	0.8	1.0	1.3	3.0	3.0	3.0
Clintafe	0	0.5	0	3.0	1.1	1.0
Clintland	0	0	0	1.5	0.8	1.3
Markton	0	1.3	2.0	1.5	0	0.7

[a] "0" indicates no telial formation; "1", scant telia; "2", moderate telia; "3", abundant telia.

younger leaves. Differences were greatest at high temperatures and with varieties exhibiting the most variation at different stages of growth. The resistant varieties and the highly susceptible variety Markton showed little differences among leaves of different ages.

DEVELOPMENT OF TELIA.—The effect of temperature and stage of growth on development of telia (Table 2) was not so consistent from one replication to another as it was on the development of uredia, but several trends were apparent. Telia were produced earlier and in greater abundance at 25° C. in most cases. This difference was greatest with the more resistant varieties. Landhafer, Bondvic, Clintafe, Clintland and Mo. 0-205 showed a pronounced development of telia at 25° C., and very little or none at 15° C. Markton and Cherokee, possibly the 2 most susceptible varieties, exhibited somewhat greater development of telia at the low temperature than at the high temperature. Marion and Appler, also susceptible, showed only slightly greater development of telia at 25° C.

The initial leaves of the seedling plants withered so rapidly after the appearance of uredia that reliable readings of telial development could not be obtained for the seedling stage. The 3 later stages of growth appeared to have little differential effect on telial formation at 15° C., but at 25° C. telial development was greater at the juvenile stage than in the boot or at anthesis.

Due possibly to the general paucity of telia at 15° C., few differences could be detected among leaves of different ages at the lower temperature. At 25° C. the 4 susceptible varieties, Appler, Marion, Cherokee, and Markton, showed a progressively greater development of telia from top to bottom. The resistant varieties that produced telia either showed no variation among leaves of different ages, or else exhibited their greatest telial development on the top leaves with progressively less development on older leaves.

DISCUSSION.—Stakman, Hart, Postigo, and Goto (8) have recently emphasized the importance of testing selections of wheat in the adult stage for reaction to specific races of stem rust under controlled conditions. Such testing is extremely difficult or impossible to carry out in the field, and consequently must be performed in the greenhouse. Among the 11 varieties of oats tested to a single race of rust the influence of temperature was pronounced on some; other varieties reacted differently at different stages of growth. Landhafer was clearly more resistant at 15° C. than at 25° C. regardless of stage of growth. The reaction of Mo. 0-205, on the other hand, was influenced primarily by stage of growth, although its reaction in the juvenile stage was influenced somewhat by temperature. Thus the stage at which a plant acquires "adult" resistance may vary with temperature. In Mo. 0-205 resistance developed between the seedling and juvenile stages at 15° C., and between the juvenile and boot stages at 25° C. However, the possibility that temperature operates on the pathogen rather than on the host is not excluded. High temperatures might favor the development of the pathogen in such a way that it is able to parasitize plants in the juvenile stage which it would be unable to attack at low temperatures. Consequently the phenomenon referred to as adult plant resistance may be a complex relationship of host and parasite interacting with temperature and other environmental factors. This can be determined only by further experiments.

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HESSIAN FLY IN RELATION TO THE DEVELOPMENT OF CROWN AND BASAL STEM ROT OF WHEAT [1]

Michael G. Boosalis

SUMMARY

Hessian fly, *Phytophaga destructor* (Say), has recently become an increasingly important factor in production of wheat in several areas of Nebraska. Results of this research indicate a relationship between Hessian fly injury and development of crown rot.

Injury inflicted to the host by the feeding larvae renders affected tissues vulnerable to attack by soil microorganisms which cause crown rot. The predominant organisms associated with the insect damage were saprophytic fungus species of *Helminthosporium* and *Fusarium*, and several nonpathogenic species of bacteria. Although healthy and deteriorated crown tissue had a similar microflora, a substantially higher percentage of the diseased tissue yielded isolates of fungi and bacteria.

Symptoms on Hessian fly infested plants with crown rot differed only in degree of severity from symptoms on infested plants free of crown rot. Stunting, delay of maturation, and grain injury were more pronounced on the infested wheat with deteriorated crowns.

The syndrome of Hessian fly infested plants with crown rot was similar to that of plants damaged solely by parasitic fungi.

Crown and basal stem[2] rot incited by fungi is one of the most important diseases affecting wheat production in many areas of Nebraska. Investigators (1, 2, 3, 5) report that the severity of root diseases may be aggravated and extended by various extrinsic factors acting on the host to reduce its resistance to the attacks of soil inhabiting microorganisms. The recent increase of Hessian fly, *Phytophaga destructor* (Say), in the southwestern part of the state correlates with the increase in incidence of crown rot.

Field observations made in 1951, 1952, and 1953 disclosed a relationship between Hessian fly injury and crown rot manifested by the deterioration of the infested tissue and by the stunting of the plant. Heretofore, this relationship has been overlooked, probably because the diseased host tissue was frequently free of insects at the time of inspection; consequently, the damage was diagnosed as being caused entirely by root rot organisms. In those instances where the insect was observed on the infected crown, the injury was usually ascribed only to the insect.

The purpose of the present research was to test the validity of the conclusion derived from field observations indicating a relationship between Hessian fly and crown rot.

MATERIALS AND METHODS.—The field soil used was a composite mixture of soils from 6 wheat fields in southwestern Nebraska. Krilium and ammonium sulfate (21 per cent nitrogen) were incorporated at concentrations of 0.10 and 0.01 per cent, respectively, of the weight of the soil having less than 20 per cent water. One-half the soil for each experiment was sterilized 2 hours at 15 lbs. pressure on 2 consecutive days.

In 1 series of studies the Hessian fly susceptible variety Cheyenne and the resistant strain Cheyenne × Ponca (C.I. 502426) were grown separately in 6-in. pots with 4 plants per pot. All seed used in this study was disinfected internally by the hot water seed treatment method for controlling loose smut of wheat and surface disinfection was accomplished by immersing the seed for 3 minutes in a 1-1000 solution of mercuric chloride. The pots for treatments involving sterilized soil were maintained in a clean draft-free area in the greenhouse in order to avoid as much contamination as possible.

In the second series of studies the sterilized soil was maintained free of fungi and bacteria, other than the bacteria carried by the Hessian fly larvae, by growing the plants in 2-quart glass jars. These jars containing soil comprised of equal amounts of the above mentioned field soil and washed sand were sterilized as described for the pot series. Each jar was plugged with a rubber stopper, 2¾ in. diameter and ½ in. wide, and fitted with 3 evenly spaced pyrex glass tubes inserted through the diameter plane of the stopper. The center tube, 0.5 in. × 3 in. extended about 1 in. below the surface of the soil and about 0.5 in. above the rubber stopper. One aseptically grown seedling of the variety Cheyenne was transplanted into each center tube. One of the 2 side tubes, ¼ in. in diameter and extending 7 in. above the stopper, reached within about 1 in. of the bottom of the jar. The other side tube penetrated about 3 in. below the surface of the soil. The side tubes afforded a means for aerating, infesting, and watering the soil. All surface areas of the rubber stopper were sealed with paraffin wax and the 3 tubes were plugged with cotton which was thoroughly dusted with sulphur every 2 weeks during the course of the experiment to avoid contamination of the soil. The transplanted seedling was guided along the side of the center tube so that it easily penetrated the space between the cotton plug and the glass surface. Since water evaporation from the enclosed soil was slight, it was necessary to water the soil in the

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[2] Unless otherwise indicated, crown whenever used in the text includes basal stem.

jars only about every 3 weeks. Each jar was also watered with 75 ml. of sterile Hoagland's complete nutrient solution during the seeding and the boot stages of plant development. By this method of growing plants the sterilized soil in about 80 per cent of the jars remained free of organisms except for bacteria carried by the larvae. Those jars found to be contaminated with fungi by plating-out samples of soil on potato-dextrose agar were discarded.

A standard procedure was devised for infesting plants with Hessian fly. The flaxseeds were obtained from infested plants collected in southwestern Nebraska, thoroughly washed in fast running tap water, and then incubated at 80° F. on filter paper saturated with water. In about 15 days flies emerged and laid eggs on leaves of caged wheat plants that were stooling. All the eggs on half the tillers of each plant were excised and all but 3 were removed from each of the remainder. After the plants were incubated for about 14 days at 80° F., larvae emerged and migrated to the basal stem and crown area of the host. At this time, the infested as well as the non-infested wheat was moved to a greenhouse kept at about 65° F. Feeding larvae developed into pupae in about 4 weeks. Subsequently, the plants were transferred to a greenhouse maintained at 40° F., and hardened for about 7 weeks. Two additional weeks of hardening were completed in a cold chamber provided with fluorescent lights and kept at about 32° F.

Observations on crown rot in the pot and jar series were made when plants were in the flowering stage and in the early boot stage, respectively. Isolations from wheat were made by washing the tissue in running tap water for 24 hours, followed by surface disinfection in a 1-1000 solution of mercuric chloride for 1 minute, and finally rinsing in sterile distilled water and plating out in potato-dextrose agar. A treatment similar to that described above was used to surface disinfect 2-week old larvae and flaxseed. To determine whether the surface disinfected larvae and flaxseed harbored fungi and bacteria internally, they were macerated and transferred to potato-dextrose agar. The external microflora of desiccated larvae and flaxseeds was isolated by rinsing the carcasses in sterile distilled water before placing them on potato-dextrose agar.

Many of the fungus and bacterial isolates from diseased crowns of wheat growing in the field and in 6-in. pots and many of the bacterial isolates from larvae and flaxseed were tested separately and in various combinations for pathogenicity on Cheyenne wheat grown in the greenhouses at 22° C. and 29° C. The fungus inoculum was grown at 27° C. for 14 days on sterilized soil medium composed of equal amounts of field soil and washed sand. Corn meal at the rate of 1 per cent of the weight of the soil was added to the substratum. The soil in each pot was infested with about 160 gm. of the fungus isolate being tested. Each treatment, consisting of 10 surface disinfected seeds planted in 6-in. pots containing infested sterilized soil, was replicated 4 times. The above procedure for testing fungus isolates was also used to test the pathogenicity of bacterial isolates. In this case, however, the bacteria were grown in potato-dextrose broth for 4 days before infesting each pot with about 30 ml. of the inoculum.

The series of studies made in 6-in. pots included 4 replicated experiments using Cheyenne and Cheyenne × Ponca wheat. The 4 treatments in each experiment included growing the Hessian fly infested plants in 1) sterilized soil; 2) unsterilized soil; and treatments 3 and 4 were similar to 1 and 2 respectively except that the plants were insect-free. Each treatment was replicated 30 times, representing a total of 120 plants. To determine whether hardening was a factor in the development of crown rot on infested plants, ½ the plants, 60, of each treatment were infested after the wheat was hardened and the remainder were infested before hardening.

Only the variety Cheyenne was grown in the jar series and the infestation was made after the plants were hardened. In addition to the 4 treatments described for the pot series, a fifth treatment consisted of growing Hessian fly infested plants in sterilized soil infested with a composite mixture of 6 fungus isolates obtained from rotted crowns of Hessian fly infested plants growing in the field (Table 1). The 6 isolates included 3 species of *Helminthosporium*, 2 species of *Fusarium*, and 1 isolate of *Rhizoctonia solani*. The experiment in this series was repeated twice.

EXPERIMENTAL RESULTS.—*Symptoms.*—The characteristic symptoms on Cheyenne wheat infested with Hessian fly before and after hardening and grown in sterilized or unsterilized soil were similar in many respects to those described by McColloch (4). Infesta-

TABLE 1.—*The effect of Hessian fly on the severity of crown rot of Cheyenne wheat infested after hardening and grown in pots and in 2-quart jars in the greenhouse*

Treatment	Percentage of plants infected with crown rot[a]	
	Severe infection	Light infection
Pot Series		
1. HFI grown in UST soil[b]	54	28
2. HFI grown in ST soil	20	45
3. NI grown in UST soil	0	20
4. NI grown in ST soil	0	15
Jar Series		
1. HFI grown in UST soil	65	25
2. HFI grown in ST soil	0	50
3. NI grown in UST soil	0	30
4. NI grown in ST soil	0	20
5. HFI grown in ST infested soil	60	30

[a] Average percentage infection from 15 replicated pots of 4 plants each and 22 replicated jars of 1 plant each for each treatment. Similar results were obtained when the experiments were repeated 3 times and twice on the pot and jar series, respectively. The results in the parallel pot series where infestation was made before the plants were hardened were comparable to the above.

[b] HFI indicates Hessian fly infested plants; UST indicates unsterilized soil; NI indicates noninfested plants; ST indicates sterilized soil.

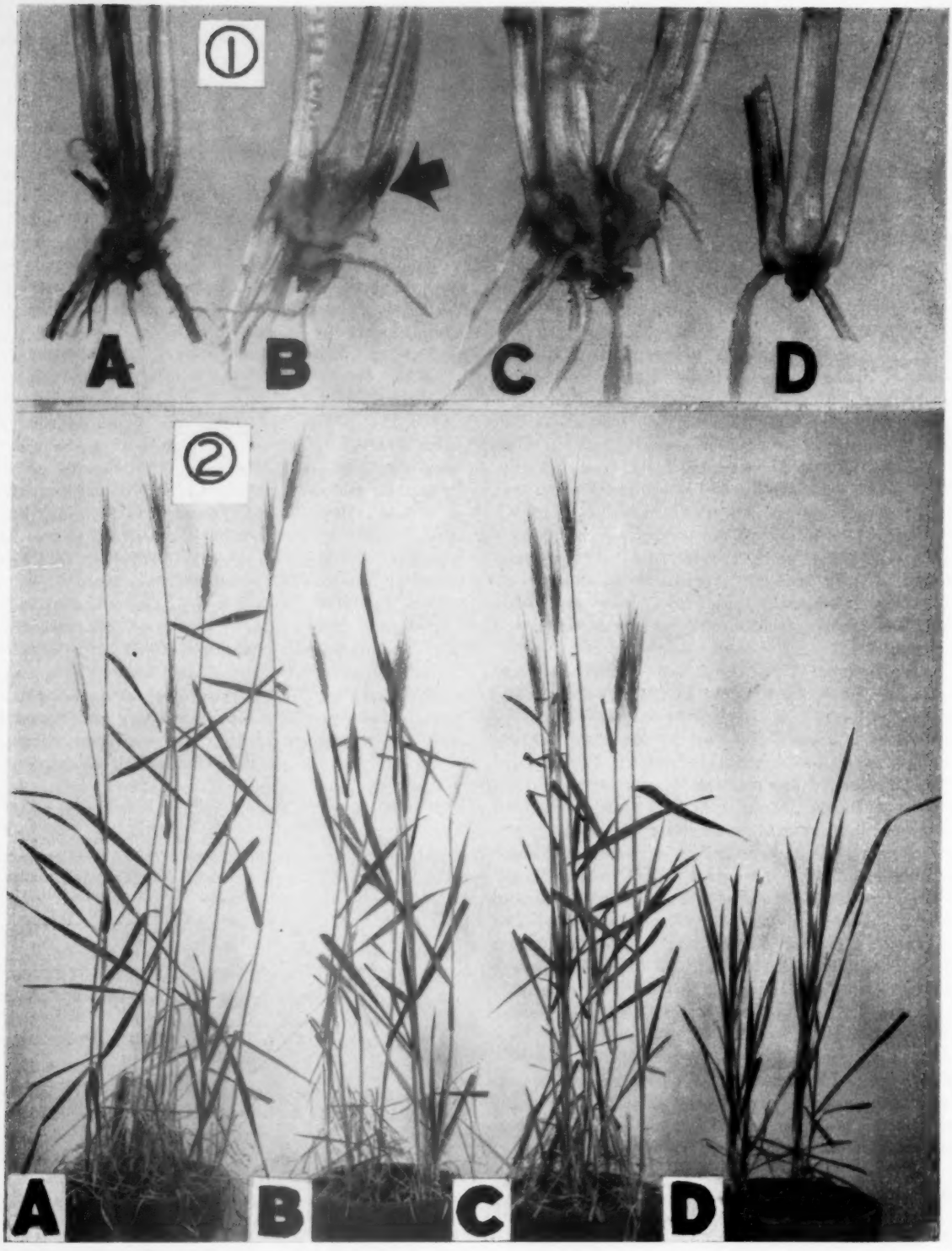

FIG. 1–2. Fig. 1. Longitudinal sections of Cheyenne wheat culms showing relationship between Hessian fly injury and the development of crown rot. A) Hessian fly infested plant grown in unsterilized soil, note severe deterioration of the crown tissue; B) Hessian fly infested plant grown in sterilized soil, arrow points to flax seed; C) Insect-free plant grown in sterilized soil; D) Insect-free plant grown in unsterilized soil.—Fig. 2. Stunting of Cheyenne wheat by the concurrent action of Hessian fly injury and crown rot microorganisms. A) Insect-free plants grown in sterilized soil; B) Hessian fly infested plants grown in sterilized soil; C) Insect-free plants grown in unsterilized soil; D) Hessian fly infested plants grown in unsterilized soil, note severe stunting of plants and delay of heading.

tion attained in this investigation, however, was not so severe as to inhibit development of the central stalk of young plants nor did the infestation prevent tillering.

Infested plants in the pot and jar series with severely deteriorated crowns could be readily differentiated from insect-free plants grown in sterilized or unsterilized soil. In contrast to the white healthy crown of insect-free plants a dark brown discoloration permeated the crown tissue of many Hessian fly infested plants, especially the infested wheat grown in unsterilized soil (Fig. 1). The roots of plants with a deteriorated crown were relatively free of discoloration and lesions and were comparable to roots of noninfested plants. The average height of 300 tillers from 100 plants with severely diseased crowns was about 5 in. shorter than insect-free plants (Fig. 2). The pronounced injury to host area near the ground level was also reflected in the length of the heads which were about 1 in. shorter than those of the control treated plants. Crown rot on the infested plants was also responsible for retarding grain maturation by 7–10 days and for the severely shrivelled, light seed.

The crown tissue of a high percentage of Hessian fly infested plants grown in sterilized soil and a relatively low percentage of the same tissue of infested wheat grown in unsterilized soil were light brown. The discolored tissue, apparently induced by insect injury alone, was superficial and did not extend deep into the affected tissue like that of severely rotted crowns of the majority of infested plants in unsterilized soil (Fig. 1). Plants with slightly rotted crowns were not so severely stunted as plants with extensively rotted crowns; on the basis of 150 plants, the average height of 300 tillers was about 2 in. less than that of the control wheat. The length of heads of infested plants with relatively healthy but discolored crowns was about 0.5 in. less than the heads of insect-free plants. Maturation of grain of the same plants was delayed 3-4 days and the resulting seed was only slightly shrivelled.

Only a few wheat plants of the Hessian fly resistant strain Cheyenne × Ponca receiving all treatments listed for the pot series (Table 1) became severely infected with crown rot.

Effect of insect injury on development of crown rot.—The highest percentage of plants severely infected with crown rot in the 2 series of studies resulted from treatment 1 in which infested Cheyenne was grown in unsterilized soil and from treatment 5 of the jar series in which infested plants were grown in sterilized. infested soil (Table 1). A heavy infection of crown resulting in deterioration of the parasitized tissue was found on over 50 per cent of the plants infested before or after hardening and receiving treatment 1 or 5. A lighter crown rot that did not produce a marked decay of the infested tissue was observed on less than 30 per cent of the wheat included in treatment 1 or 5. Although the remainder of the plants in the same 2 treatments, constituting less than 20 per cent of the total, were infested with Hessian fly, they did not develop symptoms of crown rot.

Not more than 20 per cent of the Cheyenne wheat in the pot series infested before and after hardening and grown in sterilized soil, treatment 2, were severely infected in the crown (Table 1). In the comparable treatment of the jar series none of the crowns was heavily infected. The highest percentage of plants, 45 and 50, included in treatment 2 of the pot and jar series respectively had crowns that were lightly infected.

Most of the plants, 70–85 per cent, in the pot and jar series receiving treatments 3 and 4 in which noninfested plants were grown in sterilized and unsterilized soil respectively did not develop symptoms of severe deterioration of the crowns. Similar results were obtained with the Hessian fly resistant variety Cheyenne × Ponca receiving all 4 treatments listed for the pot series of experiments (Table 1).

Microflora of Hessian fly larva, flaxseed and crown tissue of infested and noninfested plants.—Bacteria were detected in 40 per cent of the isolations of larvae from Cheyenne and Cheyenne × Ponca wheat grown in sterilized and unsterilized soil. Over 60 per cent of the bacterial colonies were white and the rest were predominately yellow.

Isolations from the internal portion of the flaxseeds yielded white and yellow bacteria at the same ratio as that found on the inside of the larvae.

Isolations from the external parts of the body yielded both fungi and bacteria from about 50 per cent of the flaxseeds collected from severely rotted crowns. About 60 per cent of the fungus isolates were species of *Helminthosporium* whereas *Fusarium* spp. were recovered from 30 per cent of the flaxseeds. *Penicillium* spp., *Alternaria* spp. and *Rhizoctonia solani* were isolated from nearly 5 per cent of the flaxseeds. Unidentified white and yellow colonies of bacteria were also found on 80 per cent of the flaxseed carcasses.

About 25 per cent of the flaxseeds from crowns with incipient symptoms of rotting were externally infested with fungi, whereas white and yellow species of bacteria were discerned on over 50 per cent of the insects' bodies. The predominant fungi occurring on the surface portion of the flaxseed were species of *Helminthosporium* and *Fusarium*.

Less than 20 per cent of the flaxseeds from Cheyenne and Cheyenne × Ponca wheat free of crown rot were externally infested with fungi. *Helminthosporium* spp. and *Fusarium* spp. again were the principal fungi isolated here. Bacterial colonies similar to those described previously were found on about 40 per cent of the flaxseeds.

The percentage of rotted crowns of Cheyenne, infested before or after hardening, infected with microorganisms was considerably higher than the percentage of infested flaxseeds from the same host. Over 90 per cent of 200 severely rotted crowns yielded isolates of fungi. *Helminthosporium* spp. constituted nearly

TABLE 2.—*Kinds of fungi and frequency of isolation from crowns of Cheyenne wheat infested and noninfested with Hessian fly after hardening and grown in pots in sterilized and unsterilized soil*

Source of isolates	Percentage of plants infected with fungi[a] Helmin. spp.	Fusarium spp.	Other[b] fungi
1. Severely rotted crown tissue of HFI grown in ST soil[c]	60	20	10
2. Moderately rotted crown tissue of HFI grown in ST soil	15	15	5
3. HFI free of crown rot grown in ST soil	10	15	0
4. Same as 1 except grown in UST soil	70	20	10
5. Same as 2 except grown in UST soil	10	15	5
6. Same as 3 except grown in UST soil	10	20	0

[a] Percentage is based on 200 crowns from 200 plants.
[b] Other fungi included: *Alternaria* spp., *Rhizoctonia solani*, and *Penicillium* spp.
[c] HFI: Hessian fly infested plants; ST and UST: sterilized and unsterilized soil respectively.

70 per cent of the fungus isolates and *Fusarium* spp. were obtained from about 20 per cent of the infected tissues (Table 2). Other fungi isolated from about 10 per cent of crown tissues included *Penicillium* spp., *Pythium* spp., *Rhizoctonia solani* and *Mucor* spp. Approximately 30 per cent of the plated out crown tissue was infected with white and yellow types of bacteria.

Microflora similar to that occurring on the severely rotted crowns of Cheyenne was found on the slightly rotted and the healthy crowns of Cheyenne and Cheyenne × Ponca wheat. In contrast to over 90 per cent of the crowns infected with fungi and bacteria, only about 30 per cent of the moderately rotted crowns and only a few of the healthy crowns yielded microorganisms (Table 2).

Pathogenicity tests of bacterial and fungus isolates.—The isolates tested for pathogenicity on Cheyenne wheat in the greenhouse at 22° and 29° C. included 20 white and 20 yellow bacterial cultures from larvae, flaxseeds, and from severely rotted crowns; 50 isolates of *Helminthosporium* spp. and an equal number of *Fusarium* spp; 2 isolates of *Rhizoctonia solani* from deteriorated crowns.

None of the bacterial and fungus isolates per se were pathogenic to Cheyenne wheat. Plants grown in the soil infested with various organisms were not damped-off and were relatively free of crown rots. It was evident from results with treatment 5 of the jar series (Table 1) that a severe type of crown rot was established on the plants that were infested with Hessian fly and grown in the soil infested with a composite mixture of 6 fungus isolates. The same isolates when tested separately and in combination did not attack the crowns of insect-free wheat.

DISCUSSION.—Results of this investigation substantiate field observations indicating a relationship between Hessian fly and crown rot. Cheyenne wheat infested before or after hardening and grown in unsterilized field soil had considerably more crown rot than infested plants in sterilized soil (Table 1). Under the conditions of this study, hardening the host before or after infestation did not affect the severity of crown rot.

The healthy crown tissue of Hessian fly infested wheat grown in soil free of fungi and those bacteria not carried by larvae, indicates that the injury to wheat caused by the insect per se does not incite severe rotting of the crown. The importance of larval damage to the host in the development of crown rot was evident when isolations were made from infested and noninfested plants grown in unsterilized soil. The type of microflora present on the rotted and healthy crown of the infested plants was similar to that on noninfested plants, although a much higher percentage of severely infected crowns was infected with microorganisms.

Proof of an existing relationship between infestation with Hessian fly and crown rot was also adduced when it was shown that none of the 150 fungus and bacterial isolates from severely rotted crowns was pathogenic on wheat free of insects. On the other hand, when plants were infested and grown in sterilized soil infested with a composite mixture of 6 saprophytic fungus isolates, the crowns were severely parasitized. It is also noteworthy that only a few of the Hessian fly resistant plants of the strain Ponca × Cheyenne became severely infected with crown rot when infested plants were grown in unsterilized soil. The resistance of this wheat was apparently due to the inability of the larvae to feed extensively on the host; consequently, the crown tissue was not sufficiently injured to pave the way for fungus invasion.

The means by which the feeding larvae weaken the host tissue to attack by the soil microflora was not investigated. It is possible, however, that in parasitizing the host the larvae render the affected tissue vulnerable to rotting by impairing the nutrition of the host, by excreting toxic metabolic products, and perhaps by weakening the plant mechanically. It is reported that the severity of injury resulting from larval feeding in the host area near the ground level is proportional to the number of insects feeding on the plant (4). Although infestations in the field may be sufficiently high to kill the host by sheer numbers of larvae feeding on the plant, there are many instances in the field where a lighter infestation results in a weakened plant that is subject to crown rot. In this study, the susceptibility of Cheyenne wheat to crown rot was substantially increased without killing the plant by infesting half the tillers of the host with 3 larvae per tiller.

The fact that the larvae were relatively free internally and externally of fungi is indicative that Hessian fly is not the vector of microorganisms inciting crown rot. This does not, however, minimize the importance of the insect in relation to the development of crown rot because the organisms which follow the injury

produced by the feeding larvae are ubiquitous and in the immediate vicinity of host tissues which are in or near the soil.

The disease syndrome caused by the concurrent action of larvae and soil microorganisms affected the plant by stunting the tillers and heads, by delaying maturation of the grain and by shrivelling the grain. Infested plants grown in soil free of fungi and bacteria other than those carried by the larvae developed symptoms similar to those described above except that the symptoms were not nearly so severe.

The symptoms on Hessian fly infested plants closely resemble those incited entirely by parasitic fungi. Unless the wheat is examined closely for Hessian fly injury the malady may be erroneously diagnosed, especially if the insect is no longer present. More care should be exercised in examining the aerial as well as the underground parts of the wheat growing in areas infested with Hessian fly in order to determine the causal agents that may be involved.

The knowledge obtained from this research may help elucidate a perplexing problem of crown rot of wheat that is a concomitant of hail in the western wheat area of Nebraska. It is commonly observed that during relatively wet falls an early and heavy stand of volunteer wheat grows in stubble fields where the current crop was hailed during the latter part of the season. McColloch (4) has stated that one of the principal sources of fly infestation is volunteer wheat. Since the stubble fields are usually not plowed until late the following spring, the fields which contain heavily infested stubble as well as infested volunteer wheat may become the focal points from which large insect populations develop and spread to the fall planted wheat in adjacent fields. In an early infestation of wheat, which frequently occurs from infested volunteer wheat, the larvae feed mainly on the crowns of the host and thereby predispose the affected tissue to rots caused by soil microorganisms. An epidemic of crown rot may follow hail, then, because during wet falls hail is a concomitant of heavy stands of volunteer wheat on which large populations of Hessian fly develop. The resulting injury from feeding larvae in turn pave the way for soil microorganisms.

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ZINC INCREASES SUSCEPTIBILITY OF BEAN LEAVES TO TOBACCO MOSAIC VIRUS [1]

C. E. Yarwood

SUMMARY

When TMV-inoculated bean leaves were dipped in 0.001 to 0.03 per cent zinc sulfate or 0.1 to 1 per cent calcium chloride solutions for 10 minutes at 10 minutes after inoculation, the number of TMV lesions formed was much greater than in control leaves treated with water. When detached bean leaves were inoculated on their upper surfaces and incubated with their lower surfaces on a substrate of 0.01 to 0.1 per cent zinc sulfate or 0.0003 to 0.003 per cent silver nitrate, the leaves were injured by the chemicals and the size of the lesions and the amount of virus present were much greater than for leaves incubated on water. When TMV-inoculated bean leaves were dipped in 0.001–0.003 per cent silver nitrate for 10 minutes, lesion formation was usually completely prevented. The same zinc treatments which increased numbers of lesions on bean decreased numbers of lesions on *N. glutinosa*.

Rumley and Thomas (1), Stanley (3), Stoddard (4), and Weintraub, Gilpatrick, and Willison (5) have demonstrated an inhibitory action of zinc on infection with tobacco mosaic virus and other viruses. Yarwood, Hall, and Nelson (10) have reported an increase in tobacco mosaic virus infection due to zinc. Data of this type could indicate a disagreement among investigators but in this case illustrate a difference due to materials and methods. Evidence which may reconcile these apparently conflicting data is presented herein.

MATERIALS AND METHODS.—The effect of zinc on tobacco mosaic virus (TMV) was tested by 3 methods on 2 hosts. Twin-leaf methods for bean (*Phaseolus vulgaris* L. var. Pinto) and half-leaf methods for *Nicotiana glutinosa* L. were used throughout. One member of each pair was treated with water and the twin was treated with zinc in water solution. In the first method, which was used in testing the effect of zinc in the substrate, both members of a pair were inoculated on their upper surfaces, washed, dried, detached, and placed with their lower (noninoculated) surfaces on the substrate. One member of the pair was placed on water in a petri dish and the other on a water solution of zinc sulfate. The leaves were incubated at about 22°C. in the diffuse light of the laboratory for 2–8 days and the necrotic virus lesions counted and/or the leaves were washed and ground and their virus content assayed by inoculating new bean leaves.

In the second method, used in testing the effect of zinc in the inoculum, the zinc sulfate was added to a ground suspension of tissue from systemically infected tobacco in 0.5 per cent dipotassium phosphate. The inoculum was applied to the carborundum-dusted leaves with a brush and the superfluous inoculum was blown off with an air blast to reduce the action of zinc on the host at the higher zinc concentrations.

In a third method, the leaves were inoculated in the ordinary way but after about 10 minutes 1 member of a pair was dipped in water for 10 minutes and the other was dipped in zinc solution. On removal from the test solutions, the leaves or leaf pieces were dried, or washed and dried, and incubated with their lower surfaces on water. In such trials, dipping in water reduced virus infection; if, therefore, the zinc effect were related to undipped controls rather than to water-dipped controls, the reported stimulation in virus activity by low concentrations of zinc would be less.

The important difference among methods is that with zinc in the inoculum or in a dip following inoculation, the zinc came in direct contact with the virus and with the host cells through which the virus entered, whereas when zinc was in the substrate, the only contact of the zinc with the virus or with the inoculated cells was through the host. With zinc in the substrate, the time of arrival and ultimate concentration of the zinc at the site of the virus was of course unknown.

The 3 types of trials were each repeated 3 or more times on bean, but were performed only once each on *N. glutinosa*. The number of lesions on the control leaves averaged about 100 per leaf.

EFFECT OF ZINC ON NUMBERS OF INFECTIONS.—In *N. glutinosa* the number of infections decreased as the concentration of zinc increased. The smallest decrease occurred when zinc was suspended in the inoculum and the greatest decrease occurred when the inoculated leaf was dipped in zinc (Fig. 1). Dips of 0.3 and 1 per cent zinc sulfate completely prevented lesion formation without apparent injury to the leaves (data not on graph).

In bean, the number of lesions increased with increasing concentrations up to 0.01 or 0.03 per cent zinc sulfate, then decreased with further increase in zinc concentration. The smallest increase occurred with zinc in the inoculum. The greatest increase occurred when the inoculated leaves were dipped in zinc, when the number of lesions with 0.01 per cent zinc sulfate was 8 times as many as on the controls. With zinc in the substrate, the lesions were increasingly difficult to distinguish as the concentration of zinc was increased and the values given for 0.1 and 0.3 per cent zinc sulfate may be subject to considerable error.

EFFECT OF ZINC ON LESION SIZE.—In all trials on *N. glutinosa* and in trials with zinc in the inoculum or in a dip on bean, no clear effect of zinc on lesion size was noted.

The size of the lesions on bean leaves on 0.01 to 0.1 per cent zinc sulfate in the substrate was usually great-

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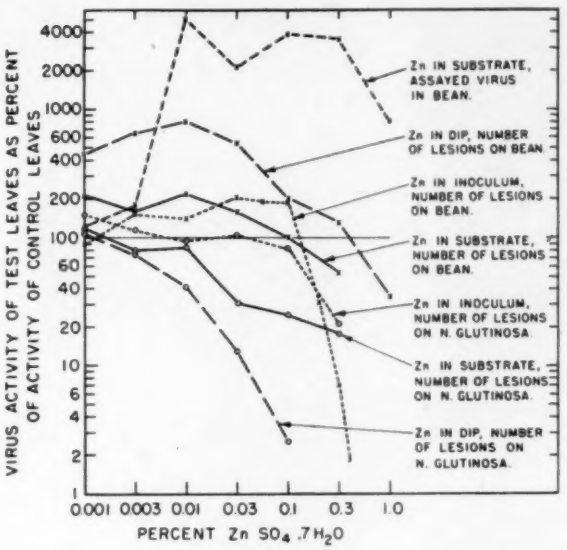

FIG. 1. Effect of zinc sulfate treatments on numbers of TMV lesions on bean and *N. glutinosa* leaves and on TMV content of bean leaves. Values above 100 indicate greater lesion numbers or virus content than for control leaves and values below 100 indicate fewer lesions than on control leaves.

er than for lesions on control leaves. In 1 representative test, the diameter of lesions on zinc-treated leaves measured 44 per cent greater than the controls for 0.01 per cent zinc sulfate, 70 per cent greater for 0.03 per cent zinc sulfate, and 108 per cent greater for leaves on 0.1 per cent zinc sulfate at 4 days after inoculation. With increasing zinc concentration and increasing chemical injury to the leaves, it became increasingly difficult to measure the size of the lesions on zinc-treated leaves. With 0.3 and 1 per cent zinc sulfate, lesions were usually not apparent because of the severe chemical injury. At these concentrations of zinc, the leaves seemed to be killed in a few days, but they became stiffer than normal and did not decompose as did dead leaves on water. Data on lesion size are omitted from Fig. 1 because it is felt that such data are unsatisfactory, especially at the higher zinc concentrations.

EFFECT OF ZINC ON VIRUS INCREASE.—The comparative virus content of the treated and control leaves is probably the most useful index of the overall effect of the zinc treatments. However, since previous studies (7) have shown a close relation between lesion size and TMV content in bean, no virus assay was made of those treatments where no effect on lesion size was detected. Because lesion number and lesion size could not be satisfactorily measured for leaves floating on a substrate containing a high concentration of zinc, virus assay of these leaves was especially essential. With 0.01 to 0.3 per cent zinc sulfate in the substrate, the virus content of the treated leaves averaged about 35 times that of the control leaves. This increase in virus content due to treatment is greater than expected from the observed increase in number and size of lesions, and may be due to the greater virus content per unit area of infected tissue in zinc-treated than in control leaves.

EFFECT OF OTHER CHEMICALS.—The effect of $AgNO_3$, $CaCl_2$, $Ca(NO_3)_2$, $CuCl_2$, $CuSO_4 \cdot 5H_2O$, $FeCl_3$, HNO_3, H_2SO_4, $HgCl_2$, K_2CO_3, $KMnO_4$, Li_2SO_4, $MnSO_4$, $NiSO_4$, $Na_2S_2O_3$, $Pb(NO_3)_2$, $ZnCl_2$, and Zinc acetate in water as substrata or dips for TMV-inoculated bean leaves was tested in a more superficial manner than was zinc sulfate. All caused bean leaf injury with increasing concentrations and all caused increased numbers of lesions and/or increased virus content at certain concentrations. The greatest increase in number of lesions occurred with calcium chloride as a dip where the number of lesions in leaves dipped 10 minutes in 1 per cent calcium chloride averaged 23 times that of the control leaves in 2 tests. The greatest increase in the amount of virus and in size of lesions resulted from 0.001 per cent silver nitrate in the substrate, where the assayed virus averaged 112 times that of the control leaves in 2 tests, and the size of the lesions was substantially increased (Fig. 2). This same concentration of silver nitrate as a 10-minute dip of leaves at 10 minutes after inoculation completely prevented lesion formation.

EFFECT OF CHEMICALS ON CERTAIN OTHER VIRUSES.—Preliminary tests with other viruses on bean indicate that their response to chemicals is different from that of TMV. Southern bean mosaic virus, tobacco necrosis virus, and an infectious entity from apple leaves showing mosaic symptoms all gave necrotic local lesions on bean, but as yet no tendency to increased lesion size from the addition of chemicals to the substrate for detached leaves has been clearly demonstrated. This may be associated with the fact that these viruses all produce much larger lesions than those of TMV on bean.

DISCUSSION.—The reduction in number of TMV lesions following treatment of *N. glutinosa* with zinc observed in this study is in accord with previous studies by other investigators (1, 3, 4, 5) and need not be further discussed. The increase in number and/or size of TMV lesions in bean following treatment with zinc and other chemicals requires explanation. The writer suggests, first, that most reported cases of chemical effects on virus increase are due to the effect of the chemical on the susceptibility of the host rather than direct effects of the chemical on the virus. Secondly, in the specific case of TMV on bean, the virus-host association is a necrotic, incompatible one characteristic of resistance, and the effect of host vigor or chemical injury on the development of a pathogen in such an association may be fundamentally different from the effect of host vigor or injury in a compatible host-parasite association such as TMV on tobacco.

The association of TMV with *N. glutinosa* is regarded as intermediate in type between that of TMV on bean and TMV on tobacco. In support of this is the fact that TMV may become systemic in *N. glutinosa* at high temperature (2) whereas TMV is not

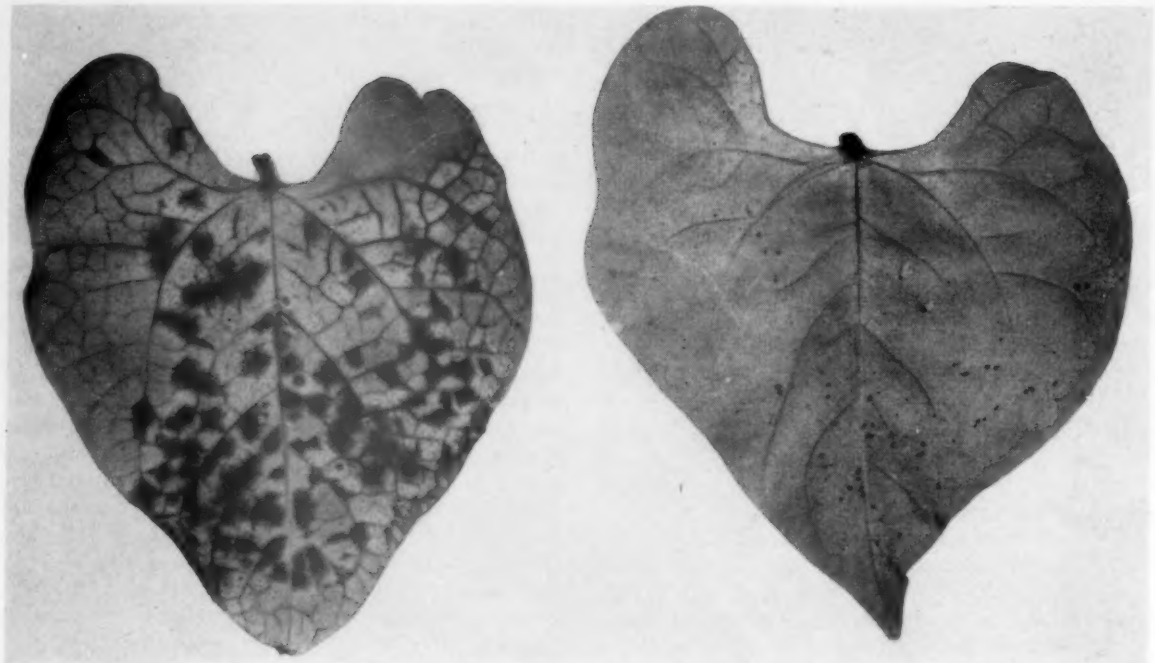

FIG. 2. Enhancing effect of silver nitrate on TMV susceptibility of Pinto bean. These opposite primary leaves were inoculated August 25 and detached. The left leaf was floated on water and the right leaf on 0.001 per cent $AgNO_3$. Both leaves were held in petri dishes at about 22°C. in natural diffuse light. Chemical injury to the right leaf (vein necrosis) is fairly distinct from the virus lesions (roundish necrotic areas). Lesion size has been greatly increased by the zinc treatment. Photographed September 4, 1952 by Victor Duran.

known to be systemic in bean. Another observation in the present study is that TMV lesions on *N. glutinosa* continue to increase in size at a fairly uniform rate up to at least 10 days after inoculation, whereas TMV lesions on greenhouse beans usually cease growth in 3 days.

With TMV on tobacco and other compatible virus-host associations, and perhaps including TMV on *N. glutinosa*, there may be an apparent correlation of host vigor and virus increase (not readily documented). On the other hand, with necrotic host-virus associations such as TMV on bean, certain treatments which reduce host vigor may also reduce resistance and thereby favor virus increase. Four such supporting examples are the increase in TMV resulting from rust infection on bean (6), the increase in TMV with decreasing carbohydrate level of bean leaves (7), the increase in TMV associated with wilting injury (8), and the increase in TMV associated with chemical injury as observed in this study.

Another possible explanation suggested to the writer is that the zinc and other chemicals may have antidoted the acquired resistance mechanism (9) in bean.

Neither hypothesis is regarded as adequately supported.

Weintraub, Gilpatrick, and Willison (5) have reported that when detached TMV-inoculated *N. glutinosa* leaves were incubated on water solutions of zinc sulfate and other chemicals, the number of TMV lesions was reduced. They suggest that this method might be useful for the testing of chemicals as virus inhibitors. In the present study, the observation of Weintraub et al with zinc has been confirmed but, in addition, it has been found that when detached TMV-inoculated bean leaves were dipped in or incubated on zinc solutions, the number and size of the TMV lesions was sometimes greatly increased in spite of severe host injury. The writer therefore questions the general utility of this method for the testing of virus inhibitors.

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A STUDY OF A NEWLY IDENTIFIED PEPPER DISEASE IN THE AMERICAS[1]

Maria Muntañola[2]

SUMMARY

Cercospora unamunoi Castellani, causing a leaf disease of pepper (*Capsicum frutescens* L.) is recorded for the first time from Argentina, Brazil, El Salvador and Venezuela. In the Western Hemisphere the disease also occurs in the southern United States.

Symptoms appear as felted brown-olivaceous patches on the lower leaf surfaces, corresponding with indefinite yellowish areas on the upper side of the blade. Affected leaves curl and drop, resulting in severe defoliation with consequent reduction of yields.

The identity of *C. unamunoi*, which has been confused with *C. diffusa* and *C. rigospora* is discussed.

The identity between *C. capsici* March. et Stey. and *C. capsici* Unamuno is confirmed on the basis of type specimens.

Colonies of *C. unamunoi* were found parasitized in some areas by a *Botrytis* here described as *B. yuae* sp. nov. related to *B. grisella* Sacc.

A leaf disease of various varieties of pepper (*Capsicum frutescens* L.) here referred to as "velvet spot," was observed in several localities in the province of Tucumán, Argentina, during the summers 1951-53. In several fields 85–90 per cent of the plants were affected, with consequent losses of 30–40 per cent of the crop. The same disease has been reported from Europe and Africa (3, 12); in America similar symptoms have been attributed to different fungi. The purpose of the present study is to establish the correct etiology of the disease and its distribution in America. This paper is also concerned with a hyperparasite which is here described as a new species of *Botrytis*.

SYMPTOMS.—Circular velvety fungus patches occur on the lower leaf surfaces, 0.3–1.5 cm. in diameter, brown-olivaceous, sometimes with an indefinite light bluish-green ring and a chlorotic halo (Fig. 1 A and B). Diffuse yellowish areas show on the corresponding upper surfaces (Fig. 1 C). The velvety aspect of the lesions is due to abundant development of the fungus which, however, does not immediately kill invaded host tissues. After some time, necrotic lesions appear generally surrounded by a narrow, elevated, tan margin, never zonate. The fungus does not attack host petioles and stems.

Affected leaves curl and drop prematurely, resulting in a severe defoliation that prevents normal development and ripening of the fruit. Badly diseased plants present a typical appearance with small, deformed, prematurely ripened fruits on the defoliated branches, and with fallen leaves, still green, covering the ground beneath.

THE CAUSATIVE FUNGUS.—The mycelium, in part superficial, invades the intercellular leaf spaces where it develops abundantly, forming substomatal stromata of varying size (average 35 × 25 μ), pale to dark brown, from which the conidiophores emerge in bundles of different sizes and number of fascicles, but always dense or very dense. Conidiophores pale olivaceous to olivaceous-brown, unbranched, continuous or sparsely septate, straight to slightly flexuous, irregularly cylindrical, frequently narrowing at the base, 22–62 × 4–6 μ (35 × 5 μ), apical end generally rounded, more or less conical, with a visible scar at the top, sometimes toothed, or with lateral conidial scars up to 15 μ well below the top (Fig. 2 G). Conidia acrogenous or pleurogenous, single or catenulate, occasionally forming chains of the same aspect as the conidiophores or acting as conidiophores (Fig. 2 K); at first hyaline and guttulate, later brown-olivaceous with a firm exospore surrounding a thin endospore. Conidia vary considerably in size and shape and can be grouped as follows: scolecospores, subfusiform or elongate-fusiform, straight or slightly flexuous, 40–80 × 5–6 μ at the base, sometimes tapering to 2 μ at the apex, continuous or several and indistinctly septate (Fig. 2 A); didymospores, subcylindrical or subfusiform, 30–50 × 4–7 μ (35 × 5), the basal cell frequently more or less inflated (Fig. 2 B); amerospores, oblong, 12–25 × 3–5 μ (Fig. 2 C). Conidial ends rounded, short-obconically-truncate, subacute, toothed or shoe-shaped, with a distinct scar at 1 end and 1–2 rarely 3 at the other. Number of septa commonly 0–1, sometimes up to 5, constricted or not at the septa.

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[2] The writer wishes to express her indebtedness to Mr. John A. Stevenson for use of material in the Mycological Collections of the Bureau of Plant Industry, for correcting the manuscript and giving many suggestions; to Dr. C. Chupp for furnishing the types of several species here discussed as well as for other help received; to Dr. P. Wygodzinsky for collecting diseased material; and to Mrs. Helen Sherwin Yu for her collaboration in the laboratory work.

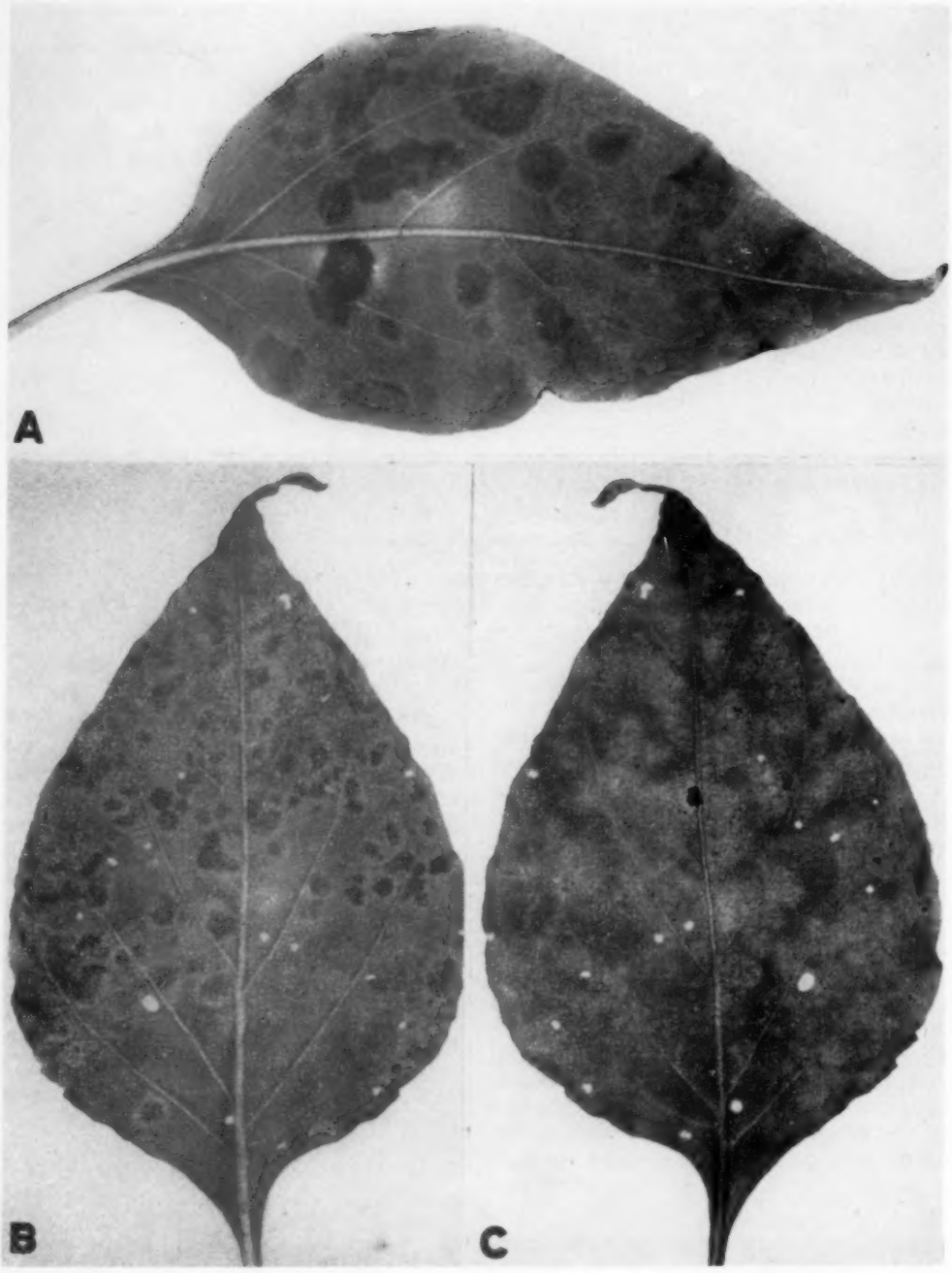

FIG. 1. A and B) velvety spots on the lower surface of a pepper leaf, most of them showing a chlorotic halo. C) chlorotic spots on the upper side of a leaf, corresponding with the fungus patches of the opposite.

Frequently a chain of conidia not yet detached can be confused with a scolecospore, but the 2 types of septation shown in the chain serve to distinguish them (Fig. 2 D). The proportion of each group of conidia varies in the different samples, but the didymospores are generally the most common. An interesting characteristic of this fungus not previously reported is the presence of a lateral acute protrusion in the conidia (Fig. 2 E), from minute to fairly well developed and distinct; the number of conidia presenting it is never abundant and varies in the different samples from 0–5 per cent.

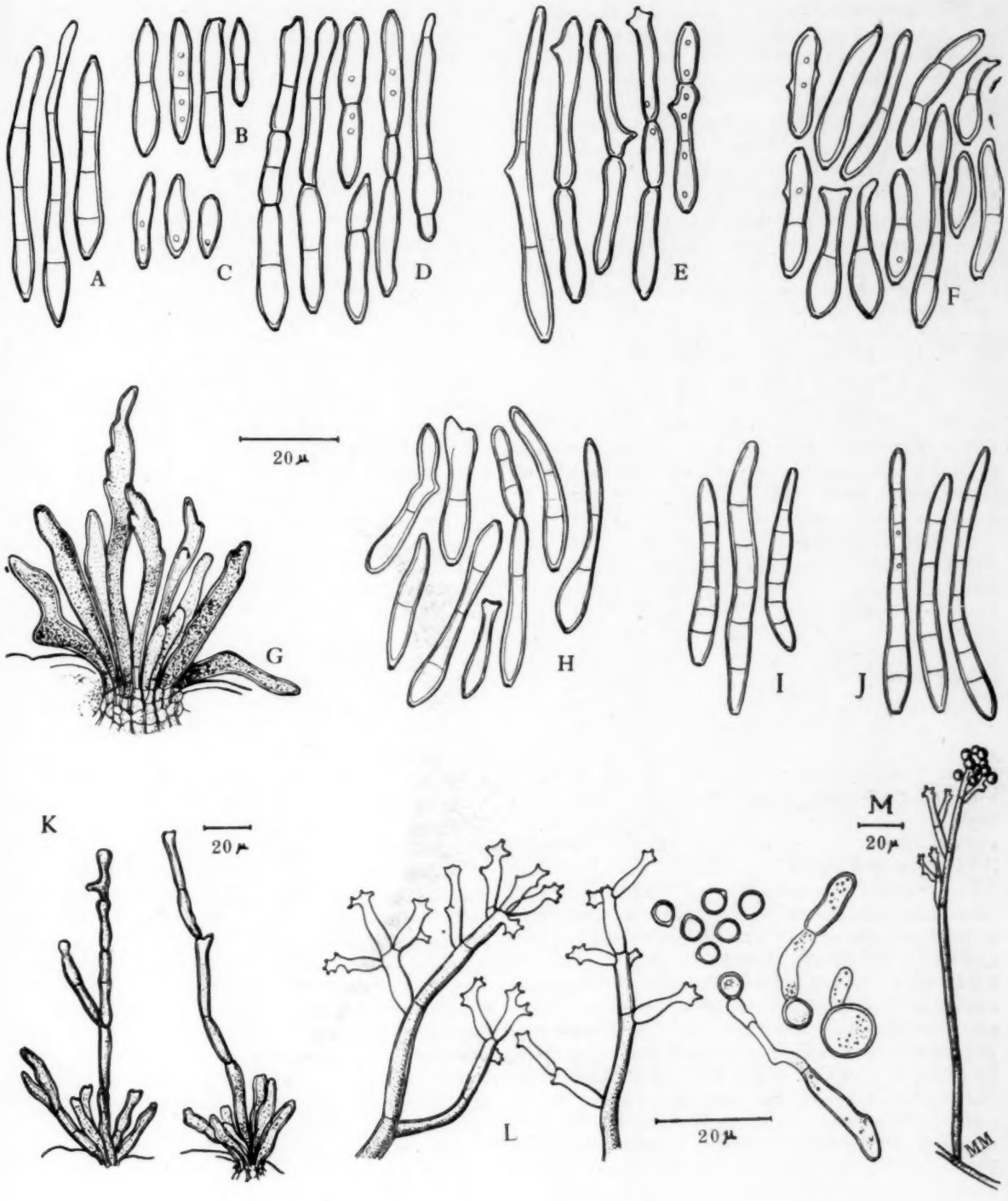

FIG. 2. A) scolecospores from fresh lesions collected at Tucumán, Arg. B) didymospores. C) amerospores. D) catenulated conidia. E) conidia of *C. unamunoi* showing a lateral protrusion. F) conidia from type specimens of *C. capsici* March. et Stey. G) conidiophores from material collected in Tucumán, Arg. H) conidia from material collected at El Salvador. I) conidia of *C. rigospora* from leaves of *Solanum nigrum*. J) conidia of *C. diffusa* from leaves of *Physalis heterophylla*. K) conidiophores and catenulate conidia of *C. unamunoi* (Tucumán, Arg.). L) detail of the apices of conidiophores of *B. yuae*, conidia and germinating conidia. M) conidiophore and clustered conidia of *B. yuae*.

In potato-dextrose agar the fungus grows very slowly. With monosporic isolations, colonies of about 2.5–3 mm. diameter were obtained after 20 days growth in the medium. They were black at the dendroid margins, and whitish in the convex, slightly pubescent centers. The mycelium is composed of hyaline to pale olivaceous, smooth-walled hyphae, 2–3 μ in diameter, with abundant short, swollen, darker, thick-walled cells. Conidia in artificial culture are scarce and smaller than those from leaf lesions.

INOCULATIONS.—The fungus was cultivated for 20 days on carrot decoction to obtain sufficient inoculum for use on a series of pepper plants. The scanty growth thus obtained was atomized, diluted in water, and sprayed on the test plants which were kept for 5 days in a moist chamber at a maximum temperature of 20° C. After 10 days, very small light-brown patches, visible only under a hand lens, but otherwise resembling those found in nature, were noted on the lower leaf surfaces. These poor results were probably due to low temperature prevailing when inoculations were made, but were sufficient to prove the pathogenicity of the organism under study, and to confirm results of inoculation studies reported by Kovachevsky (11).

FACTORS INFLUENCING THE DEVELOPMENT OF THE DISEASE.—High temperature and high humidity are factors favorable to the development of the disease. Field infection increases in rainy weather and when plants have reached maximum size, since the consequent shading helps maintain a high humidity. In certain fields the fungus was found attacking only plants under deep shade; in several others it was observed only on the lower leaves. This cannot be attributed to the age of these leaves, but to the protection from the sun received by the upper ones, because at the base of the plants there were new branches showing the disease. In the field the disease appears in epiphytotic form only when the weather is consistently warm and humid.

DISTRIBUTION AND HISTORY.—In 1926 Bensaude (2) found the same disease in the Azores, attributing it to *Cladosporium* sp. In 1929 Marchal and Steyaert (14) described the causative fungus from the Belgian Congo as *Cercospora capsici*. Unaware of this paper, Unamuno (33) in 1932 described it again from Spain under the same name. Kovachevsky (11) in Bulgaria pointed out the differences between the disease caused by *Cercospora capsici* Heald et Wolf 1911 (8) and that described by Marchal and Steyaert which he considered the same as Unamuno's organism, changing the genus of the latter fungus to *Cladosporium*. In 1948 Castellani (3) reported from Ethiopia a pepper disease caused by a fungus he considered to be Unamuno's *C. capsici*, and for which he proposed the binomial *Cercospora unamunoi*.

Several authors (17, 26, 34) have reported from Brazil a disease on *Capsicum* which although referred to *Cercospora rigospora* Atk. is apparently the same as that here discussed. *C. rigospora* was originally described as producing sooty indefinite patches on leaves of *Solanum nigrum* (1). A study of type specimens of *C. rigospora* as well as other specimens on *Solanum* spp. has been carried out, and the morphology of this organism does not agree with that of the pepper fungus. *C. rigospora* has less compact, more divergent, paler conidiophores, and regularly subcylindrical conidia, 40–60 × 3–4 μ, 3–10 septate (Fig. 2 I). On the other hand the author has seen material from Cantareira, State of São Paulo, Brazil, identified as *C. rigospora*, which was actually the same as the species discussed in the present paper.

In the Mycological Collections of the Bureau of Plant Industry at Beltsville, Maryland, typical material was found on specimens filed as *C. capsici* Heald et Wolf, the second fungus having been overlooked or referred with doubt to *C. diffusa* Ell. et Ev. This latter species was originally described on *Physalis lanceolata* (4) and although the spots that it produces resemble those of the pepper fungus, the organism is morphologically different. The conidia are subcylindrical, slightly attenuated towards the ends, mostly about 60 × 4 μ, but varying from 40–90 μ in length and 3–8 septate (Fig. 2 J).

Stevenson and Wellman (31) reported from El Salvador *C. diffusa* on *Capsicum*, which identification was accepted by Castellani (3) who attributed the differences between the fungus from El Salvador and *C. diffusa* to the variations in *Cercospora* discussed by Welles (36, 37). The author has carefully examined specimens collected by Wellman in El Salvador and found that they agree with those collected in Tucumán.

Additional *Capsicum* specimens found attacked by the fungus were from Florida, California, Texas, El Salvador and Venezuela.

Recently Miller and Taylor (16) have reported the disease as serious in a field in Union Co., Georgia, under the name *Cladosporium capsici* (March. et Stey.) Kovac.

TAXONOMY.—Because of its characteristics the proper systematic position of this fungus is not easily arrived at. Kovachevsky (11) gave the following reasons for transferring it from *Cercospora* to *Cladosporium*: the symptoms of the disease caused by it are similar to those produced by *Cladosporium fulvum* on *Lycopersicon;* development of the spots is strongly dependent on high humidity with both fungi; both are parasitized by *Botrytis;* the Capsicum fungus forms colonies in hanging drop cultures which resemble very closely those of *Cladosporium cucumerinum*.

Castellani (3) did not accept Kovachevsky's conclusions and considered the organism to belong to what he calls "*Cercospora* of the *Ragnhildiana* group" not considering *Ragnhildiana* Solheim (28) as a genus with enough characters to be maintained. Neither did he accept Kovachevsky's identification of *C. capsici* Unamuno with *C. capsici* March. et Stey., emphasizing the fact that in the illustrations given by Marchal and Steyaert the stroma is rather small. The author has studied type specimens of Marchal and Steyaert's

fungus, and they completely coincide with those of Unamuno's.

As for including the fungus in *Cercospora* it becomes as difficult as including it in *Cladosporium*. The amerospores and didymospores conform to the characters of *Cladosporium*, as do also the colonies in artificial media, while the conidiophores and scolecospores definitely relate this fungus to the *Cercosporae*.

Several genera have been proposed for these forms intermediate between *Cercospora* and *Cladosporium*. *Ragnhildiana* Solheim (28) based on catenulation of conidia and comprising species which cause diffuse velvety leaf patches, has been rejected by several authors (17, 21) who do not consider catenulation a stable enough character for generic distinction as it applies to *Cercospora*. It has been considered a synonym of *Corynespora* Gussow (23) in which conidia are also produced in chains. In *Corynespora* the conidiophores arise perpendicularly to the surface of the substratum, from subepidermal or aerial hyphae, commonly single and simple, reaching a length of 600 μ or more and diameters of 11.3 μ in the type species (35); a hyaline isthmus often connects conidia with the conidiophore, the shape of the conidia relating this genus to *Helminthosporium*. The author does not consider *Ragnhildiana* to be the same as *Corynespora*, and the Capsicum fungus could not be included in this genus.

Petrak (20) has suggested that *Ragnhildiana* could probably be included in *Chaetotricum* Sydow (32), a genus with superficial mycelium forming cobwebby mycelial leaf-patches. This has also long, commonly single conidiophores representing lateral, perpendicular branches of the mycelium. The type species, *Ch. solani* Sydow (29) also represents an intermediate form between *Cercospora* and *Helminthosporium*. Some effuse spot-producing fungi formerly considered as *Cercospora* or *Cladosporium* have recently been kept by Hughes (9) in *Passalora*.

In the Capsicum fungus the conidiophores are gathered in short fascicles and the isthmi present in *Corynespora* or the setae-like branches of *Chaetotrichum* never have been seen. The didymospores with a swollen basal cell of *Passalora* are not rare but they are always present among many other forms. The whole group of intermediate forms between *Cercospora* and *Cladosporium* needs further study, and in the present paper the Capsicum fungus is retained in *Cercospora* principally in order to avoid more new combinations until the group becomes better known.

The pertinent synonymy of this fungus is as follows:

Cercospora unamunoi Castellani. Riv. Agric. Subtrop. Trop. 42, 1-3: 20-29. 1948.

Cercospora capsici March. et Stey. Bul. Soc. Roy. Bot. Belge 61: 67. 1929.

Cercospora capsici Unamuno Bol. Soc. Española Hist. Nat. 32: 163. 1932.

Cladosporium capsici Kovachevsky Zeitschr. f. Pflanzenkrankheiten 48: 335. 1938.

Cladosporium sp. Bensaude Kew Bul. Misc. Inform. 1926, 9: 384. 1926.

Non *Cercospora capsici* Heald et Wolf Mycologia 3: 15. 1911.

HYPERPARASITE.—On leaves of the "hot" pepper varieties most of the spots were observed to be grayish instead of brown. Under a hand lens the felted patches appeared to be more or less overgrown by another quite different fungus, and by microscopic study it was possible to see that the mycelium of this second organism was growing on the tufts of *C. unamunoi*. This hyperparasite was first noted by Kovachevsky (11) who identified it as a *Botrytis* sp. and demonstrated the parasitic relation between it and the pepper fungus.

A critical examination of the species reported in literature as parasites of *Cercospora* and related genera, or associated with them, and of the material available in several North American Herbaria, has led the author to consider this fungus as belonging to an undescribed species, and the name *Botrytis yuae*, after Mrs. Helen S. Yu, is proposed for it.

Botrytis **yuae** sp. nov.

Caespitulae griseolae vel murino-griseae, ex hyphis subhyalinis vel brunneo-griseolis, septatis, 3 μ crassis formatae; caespitulas matricis omnino tegentes. Conidiophora erecta, solitaria, septata, 180–250μ longa, dichotomice vel invicem, aut nonnumquam subverticillatim ramosa; prope basim 4 μ crassa et brunneo-griseola, apicem versus pallidiora et saepe paullo attenuata; cellulis apicalibus hyalinis, subfusoideis, 10–15 $\times$ 2 μ (minimis 1.25, maximis 2.6 μ crassis), raro cuboideis, nonnumquam sparse denticulatis, denticulis sporogenis plerumque plus minusve manifestatis, acrogenis, 3–4 congregatis et verrucam formantibus. Conidia globosa, ad basim minutissime papillata, subtilissime verruculosa, hyalina, simplicia, 3–5 μ diam.

Parasitica in Cercospora unamunoi ad folia viva Capsici frutescentis.

Somewhat wooly tufts, grayish or mouse-gray in color. Hyphae subhyaline to brown-grayish, septate, 3 μ wide. Conidiophores erect, single, 180–250μ long, dichotomously or alternately branched, rarely subverticillate; brown-grayish and thicker at base, fainter colored and narrowing upwards. Each branch generally separated by a septum and branching again dichotomously, opposite or alternately in a dendroid irregular form. The upper cells hyaline, 10–15 $\times$ 2 μ (1.25 μ at the narrowest point, 2.6 μ at the widest), subfusiform, rarely elbowed, sometimes sparsely toothed throughout their length, but the denticles generally clustered 3–4 together in more or less conspicuous terminal swellings. Conidia produced on the denticles, globose, somewhat apiculate at their base, smooth to very finely rugose when observed under the oil immersion lens, hyaline, continuous, 3–5 μ diam. (Figs. 2 L and M).

Parasitic on *Cercospora unamunoi*, on living leaves of *Capsicum frutescens*. Type specimen deposited at

the Instituto Lillo, Tucumán, Argentina. Type culture at Cornell University, N. Y.

This species seems closely allied to *B. grisella* Sacc. (= *B. griseola*) (24, 27), from which, according to the original description, it differs principally in the conidiophore apices. In *B. grisella* these apices are said to be pointed and naked, while those of *B. yuae* are commonly multicuspidate. The original illustration of *B. grisella* is rather poor (25) and that given by Penzig (19) distinctly differs from *B. yuae* also in the tips of the small branches. The diameter of the spores is 5–7 μ in *B. grisella*, somewhat larger than in *B. yuae*. Furthermore, *B. grisella* has been reported growing on bark of citrus, *Prunus*, *Salix* and other woods, decayed or not (6, 24, 27), while the present fungus lives as a parasite on another fungus, a fact which appears to the author to confirm its specific standing.

B. hypophylla Ell. et Kell. (5), described as occurring on leaves of *Teucrium canadense* associated with *Cercospora ferruginea* Fckl., and also parasitic on *C. hydropiperis* (Thüm.) Speg. (= *Helminthosporium hydropiperis* Thüm.) on *Polygonum hydropiperis* (Ellis and Everhart's North Am. Fungi n. 2460), differs among other features in its conidia which are smooth, more pyriform and larger than those of *B. yuae*.

B. cercosporaecola Hara (7), a parasite of *C. kakivora*, differs in its hyaline, 13–17 × 4–5 μ conidiophores, and in its 4.4–8.8 × 3–4 μ conidia.

Rippel (22) mentioned a *Botrytis* sp. on *Cladosporium fulvum*, but he did not give any description or other detail by which it could be recognized.

Rhinotrichum canescens and *R. canescens* var. *speciosulum* Speg. (30) parasites of *Cercospora sphaeroidea* Speg. on leaves of *Cassia* sp. were considered by Linder (13) as probably belonging to *Sporotrichum*, in which they were originally described (29). The decumbent sporophores of this genus do not agree with the erect ones of *B. yuae*.

R. griseo-roseum Marchionatto (15) associated with *C. capsici* Heald et Wolf, has denticulate conidiophores which bear the conidia throughout their length. It forms greenish colonies which turn Vandyke brown (Ridgway) with age, finally becoming pinkish-gray with the reverse side yellowish or orange. All these characters separate this fungus definitely from *B. yuae*.

Verticillium cercosporae Pet. et Cif. (21) on *Cercospora* spp. on *Solanum* and *Hibiscus*, has verticillate branches.

It is interesting furthermore to mention *B. terrestris* Jensen (10), the original description of which fits well with the one of *B. yuae*. The type culture was examined, but it was found that the species does not correspond to the genus *Botrytis*.

Material studied.—Specimens numbered 126–130, 250–256 in the author's herbarium, collected in several localities in Tucumán, Argentina, on different varieties of *Capsicum frutescens* L., as well as specimens from Florida, Texas, California, El Salvador, and Brazil in the Mycological Collections of the Bureau of Plant Industry, Beltsville, Md., have been available for this study.

Type specimens of *C. capsici* March. et Stey., *C. capsici* Unamuno, *C. capsici* Heald et Wolf, *C. diffusa* Ell. et Ev., *C. rigospora* Atk., as well as diseased pepper leaves from Venezuela, have been furnished by Dr. Chupp from his *Cercospora*-type collection at Cornell University, N. Y. The culture-type of *Botrytis terrestris* has been kindly furnished by Dr. Korf, Cornell University.

Material from Argentina affected by *C. unamunoi* and *B. yuae* has been deposited in the herbarium of the Instituto Lillo, Tucumán, Arg., at Beltsville, Cornell and Harvard (Farlow).

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PATHOGENICITY OF VERTICILLIUM ISOLATES TO PEPPERMINT [1]

C. E. Horner

SUMMARY

Seventeen isolates of *Verticillium albo-atrum* from 11 hosts were infectious to peppermint. The 17 isolates were consistently separable into 3 groups by differences in symptoms, host range, and relative growth in peppermint stems. It was concluded that isolates in the 3 groups represent at least 3 strains of *V. albo-atrum* differing in their pathogenicity to peppermint.

One isolate of *V. albo-atrum* from Oregon peppermint infected the roots of all 7 plant species tested and invaded the stems of 5 of the 7 species. One isolate of *V. albo-atrum* from Indiana peppermint infected the roots of 4 and invaded the stems of 3 of the 7 plant species tested.

Peppermint is a symptomless host and potential reservoir for strains of *Verticillium* capable of causing disease in other crops. Several weeds and crop plants are hosts for strains of *Verticillium* capable of producing wilt in peppermint.

Verticillium wilt (*Verticillium albo-atrum* R. & B.) has been a serious disease of peppermint (*Mentha piperita* var. Mitcham) in Michigan and Indiana since it was first described from that area by Nelson in 1926 (4). The disease was first reported from Oregon by Boyle in 1944 (1). In 1950, Nelson, in his monograph of this disease (5), published a colored illustration of peppermint wilt in an Oregon field and reported observing the disease in Oregon and Washington in 1946.

After extensive experiments, Nelson concluded that *Verticillium* isolates from other hosts were not pathogenic to peppermint; that the *Verticillium* from mint was not pathogenic to other plants. Therefore, he considered the mint wilt fungus distinct and named it *Verticillium albo-atrum* R. & B. Var. *menthae* Nelson (5). On the contrary, Green (2) reported in 1951 that eggplant and pepper were hosts for *Verticillium* isolates from peppermint and that isolates from tomato and radish infected peppermint.

Surveys made in 1949 by workers familiar with peppermint wilt in Indiana, and general observations, indicate that the disease was not of economic importance in Oregon prior to 1950. Surveys made in 1951 and 1952 indicated increased importance of the disease and justified the present studies. Many isolations were made from stems and roots of peppermint and various weed species collected from the major mint growing areas of Oregon. *Verticillium* was isolated from roots and lower stems of peppermint not showing typical wilt symptoms, as well as from peppermint showing typical wilt symptoms. Also, *Verticillium* was isolated from various weeds in and around peppermint fields. The general presence of *Verticillium* on peppermint with and without wilt symptoms and on weed species suggested that different pathogenic strains were present. In 1952 and 1953 greenhouse experiments were conducted to test the pathogenicity of *Verticillium* isolates on peppermint. A preliminary report has been published (3).

Materials and methods. — The peppermint test-

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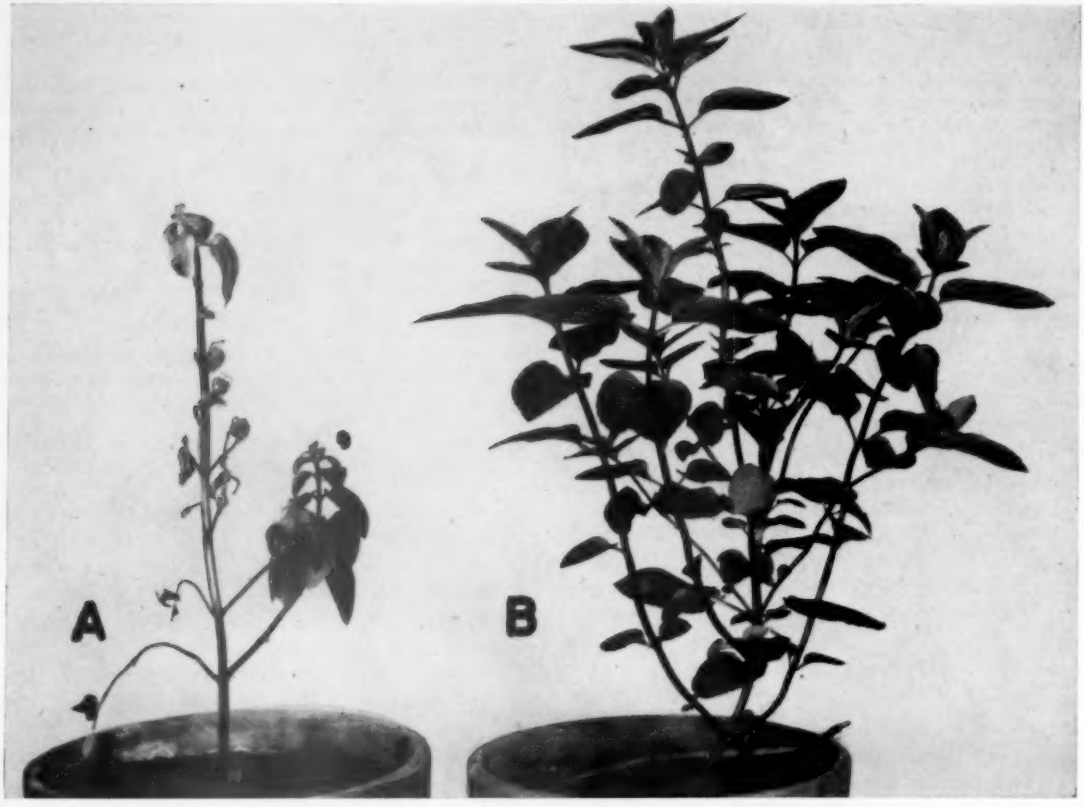

FIG. 1. Peppermint plants inoculated with *Verticillium*. A) Isolate from severely wilted mint. B) Isolate from symptomless mint. (Photograph by H. H. Millsap.)

plants were grown from cuttings taken from healthy plants and rooted in steamed sand. Seventeen cultures derived from single spore or single hyphal tip transfers maintained on potato-dextrose agar by mass transfers were used as inoculum. Seven were from peppermint and 10 from other hosts. Of the 7 from peppermint, 4 were from plants with typical wilt symptoms and 3 from plants without wilt symptoms.

Plants were inoculated by 2 methods. One series of rooted cuttings was set in steamed soil in clay pots and inoculated by wounding with a sterile scalpel then inserting fragments of mycelium on agar into the wound. The wound was covered with a pad of cheesecloth bound in place with tape. The cheesecloth was extended above the tape to serve as a wick and was wet daily for 5 days following inoculation. Only sterile agar was placed in wounds on the control plants. A second series of test-plants was exposed to infection by mixing cultures grown aseptically on whole oats with steamed soil and setting rooted cuttings in the infested soil. Control plants were grown in steamed soil to which sterile oats had been added. Five plants in each series were inoculated with each isolate. Plants were grown for 7 weeks at a mean greenhouse temperature of 22° C.

Reisolations were made to determine extent of growth of *Verticillium* in peppermint stems. Wound-inoculated plants were cut at the soil line and sections for reisolation taken from each stem at positions 2 in. below the point of inoculation and 2, 4, 6, 8 and 10 in. above the point of inoculation. Soil-inoculated plants were cut 2 in. below the soil line and sections for reisolations were taken from each stem 2 in. below the soil line, at the soil line, and 2, 4, 6 and 8 in. above the soil line.

RESULTS.—*Pathogenicity of isolates from various hosts to peppermint.*—Plants exposed to infection in infested soil exhibited striking reactions consisting of chlorosis, reddening of the leaves, and stunting. These symptoms disappeared 14–18 days after exposure to infection. Since neither wound-inoculated plants nor plants in soil to which sterile oats were added showed such symptoms, it is probable that the symptoms were due to toxic fungus by-products accumulated in the oat medium.

After 3 weeks, plants inoculated with isolates from typically wilted peppermint began to show yellowing, reddening, stunting, asymmetrical development of the leaves, and shortening of internodes, whereas plants inoculated with other isolates remained symptomless. After 7 weeks, plants inoculated with isolates from typically wilted peppermint developed typical wilt symptoms (Fig. 1, A). Plants inoculated with isolates from peppermint without typical wilt symptoms showed

TABLE 1.—*Comparison of growth in peppermint stems of 17 Verticillium isolates introduced by wound inoculation or from infested soil*

Source of isolates and grouping by L.S.D.[a]	Isolate number	Distance of growth in stem (in.)[a] Wound inoculated	Infested soil
Group I			
Peppermint with symptoms	A-1	10.0	9.6
Peppermint with symptoms	H-1-H	9.6	9.6
Peppermint with symptoms	P-1-P	9.6	8.0
Peppermint with symptoms	B-11-B[b]	8.2	8.4
Group II			
Peppermint without symptoms	H-N-S	4.4	5.2
Peppermint without symptoms	H-2	4.0	5.2
Peppermint without symptoms	B-2	4.0	6.0
Group III			
Snowberry (*symphoricarpos albus*)	M-3	2.0	3.2
Maple (*Acer rubrum*)	M-1-Y[c]	2.0	3.2
Tomato (*Lycopersicon esculentum*)	W-9[d]	2.0	2.8
Mallow (*Malva rotundifolia*)	L-3	1.6	3.2
Potato (*Solanum tuberosum* var. White Rose)	P-1-M	1.2	2.4
Raspberry (*Rubus occidentalis* var. Washington)	B-1-P	0.8	2.8
Burdock (*Arctium minus*)	B-2-D	0.8	3.2
Canada thistle (*Cirsium arvense*)	C-1	0.8	2.0
Lamb's quarters (*Chenopodium album*)	J-1	0.4	3.2
Peony (*Paeonia albiflora*)	P-1-Y[c]	0.4	3.2

[a] Least significant difference at .05 for wound inoculated series = 1.56 in.; for infested soil series = 1.55 in.
[b] Supplied by R. J. Green, Purdue Univ.
[c] Supplied by Roy Young, Oregon State College.
[d] Supplied by Stephen Wilhelm, Univ. Calif.

only slight yellowing of the lower leaves or were symptomless (Fig. 1, B). Plants inoculated with isolates from hosts other than peppermint showed no visible symptoms.

The relative growth of the 17 isolates in peppermint stems determined by reisolation is shown in Table 1. All isolates grew in peppermint stems but differences in the distances of growth in the stems were striking. Significant differences in the distances of growth segregated the isolates into 3 groups which correspond to 3 groups established by field symptoms and by hosts.

Pathogenicity of isolates from peppermint to other hosts.—Verticillium isolates A-1 and B-11-B from severely wilted peppermint in Oregon and Indiana, respectively, were used to inoculate tomato, peppermint, and the 5 weed species listed in Table 2. The test-plants were grown from seeds or rhizomes in steamed sand and transferred to infested soil in clay pots. After 9 weeks at a mean greenhouse temperature of 23° C. plants were removed from the pots and the soil washed from the roots. Results of inoculations from roots and stems of each plant are in Table 2.

The Oregon isolate (A-1) infected the roots of all species tested, grew at least 2 in. up the stems of all the species except burdock and mallow, and caused typical wilt symptoms on Canada thistle, lamb's quarters, peppermint, and tomato but not on the other hosts. The Indiana isolate (B-11-B) infected the roots of Canada thistle, lamb's quarters, peppermint, and tomato, but not burdock, pigweed, or mallow; it grew up the stems of Canada thistle, peppermint, and tomato and caused typical wilt symptoms only on Canada thistle and peppermint.

DISCUSSION.—*Verticillium* isolates from a wide variety of hosts infected peppermint. Isolates of *Verticillium* from peppermint from Oregon infected 6 other

TABLE 2.—*Comparison of pathogenicity of 2 Verticillium isolates from peppermint to other hosts*

Host	Number of plants inoculated with each isolate	Isolate A-1[a] Number plants yielding Verticillium from Roots	Stems[c]	Isolate B-11-B[b] Number plants yielding Verticillium from Roots	Stems[c]
Burdock (*Arctium minus*)	5	3	0	0	0
Canada thistle (*Cirsium arvense*)	5	5	5	5	5
Lamb's quarters (*Chenopodium album*)	5	5	4	5	0
Mallow (*Malva rotundifolia*)	5	2	0	0	0
Pigweed (*Amaranthus retroflexus*)	5	5	2	0	0
Peppermint	10	10	10	10	10
Tomato	10	10	5	7	4

[a] From severely wilted peppermint in Oregon.
[b] From severely wilted peppermint in Indiana.
[c] Data in this column from reisolation points on the main stem 2 in. above the soil line.

plant species tested, and isolates from peppermint from Indiana infected 4 of the 6. The criteria of symptoms, host preference, and distances of growth of the isolates in mint stems were in agreement in placing the isolates into 3 groups. From this, it is concluded there are at least 3 strains of *Verticillium* that differ in pathogenicity to peppermint. However, all the isolates infected mint and hence may be considered potentially pathogenic to mint. This is not in agreement with the statements of Nelson (5) that *Verticillium* from other hosts did not infect mint. The discrepancy between the results reported here and those of Nelson can be explained by the fact that he interpreted absence of symptoms as evidence of lack of infection. Also, it should be noted that although 3 of the isolates tested by Nelson were from the same hosts as 3 used in these tests (maple, raspberry, and tomato) they were from a different section of the country. The only non-mint host on which both this investigator and Nelson tested mint isolates was tomato.

Recently Keyworth (4) has proposed that the site of differential resistance of plants to *Verticillium* is in the roots and demonstrated that stems of hop varieties of low and high resistance show equal resistance to both mild and severe strains of *Verticillium*. Data presented here indicate that peppermint stems differ in their resistance to mild and severe strains of *Verticillium* and that resistance is not localized in the roots.

Verticillium from maple, peony, potato, tomato, and raspberry can infect peppermint. Thus, peppermint can serve as a symptomless host for perpetuating *Verticillium* strains that cause severe disease in other crops. Both weeds and certain crop plants are infected by strains of *Verticillium* that cause a wilt in peppermint and in the absence of peppermint can serve as hosts for the maintenance or build-up of *Verticillium* strains that cause peppermint wilt.

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THE EFFECT OF PURINES, PURINE ANALOGS AND RELATED COMPOUNDS ON THE MULTIPLICATION OF TOBACCO MOSAIC VIRUS [1]

I. R. Schneider

SUMMARY

Purines, purine analogs, and related compounds were screened for their effect on TMV multiplication in tobacco leaf disk culture. The following compounds were most effective in inhibiting virus formation: 8-azaguanine, 8-azaadenine, 2-azaadenine, and 2,6-diaminopurine. The amount of virus formed decreased with increasing concentrations of compound supplied. Other purine analogs that significantly inhibited TMV multiplication with little or no observable injury to leaf disks are: benzimidazole, 6-nitro-benzimidazole, 2-ethyl-5-methylbenzimidazole, thioguanine, 2-methylthioadenine, and 2-iodoadenine.

Neither 8-azaadenine nor 2,6-diaminopurine completely stopped TMV multiplication throughout the disks during a 6-day period, but they greatly reduced the rate of virus multiplication.

In all attempts to reverse the inhibition of virus multiplication by either 8-azaadenine or 2,6-diaminopurine, adenine caused complete reversal, and adenosine and adenylic acid were highly active. Guanine demonstrated appreciable reversing activity, but in contrast to adenosine and adenylic acid, guanosine and guanylic acid were inactive. The compounds xanthine, hypoxanthine, and p-aminobenzoic acid showed little or no reversing activity. These reversal experiments suggest that both 8-azaadenine and 2,6-diaminopurine inhibit the utilization of a substrate, possibly adenine, involved in the formation of TMV. Adenosine, adenylic acid and guanine may apparently be reversibly converted to the inhibited substrate in the host, but guanosine, guanylic acid, xanthine, hypoxanthine, and p-aminobenzoic acid are apparently not subject to such conversion.

A limited number of purines and purine analogs have been tested for their effect on plant virus diseases. Matthews (2,3,4) found that guanazolo (8-azaguanine), the triazolo analog of guanine, when sprayed on leaves of tobacco or *Nicotiana glutinosa* plants, reduced the number of local lesions resulting from inoculation with lucerne mosaic virus. The same treatment delayed or prevented symptom appearance. He found that spraying with 8-azahypoxanthine, the triazolo analog of hypoxanthine, was effective in reducing the number of local lesions in beans that were inoculated with lucerne mosaic virus. Mercer, Lindhorst, and Commoner (5) reported that 8-azaguanine inhibited tobacco mosaic virus (TMV) multiplication 60 per cent in tobacco leaf disks cultured in nutrient solution for 260 hours after inoculation. They also reported 80 per cent inhibition of TMV multiplication when 2,6-diaminopurine was supplied, confirming a recent report by Ryzhkov and Marchenko (7).

In the following pages are presented the results obtained in screening purines, purine analogs, and related compounds for their effect on TMV multiplication in tobacco leaf disks, together with further studies with 2 purine analogs, 8-azaadenine and 2,6-diaminopurine,[2] that strongly inhibited TMV formation.

MATERIALS AND METHODS.—The screening procedure (6, 8) employed 14 disks per sample (approximately 0.5 gm. fresh tissue) cut from leaves of *Nicotiana tabacum* var. Trelease approximately 24 hours after inoculation. The disks were floated on aqueous solutions of the chemicals in Petri dishes 5–6 days, with daily discarding of the solutions, rinsing of disks with distilled water, and replacement of the solutions. The relative concentration of TMV in each sample was determined by using a chloroform-water emulsion assay procedure (9), with minor modifications. These changes include the following: the homogenized leaf disks with the combined rinsings are brought to the mark of a 5 ml. volumetric flask. The suspension is well mixed and then transferred to a 10 ml. flask for the chloroform emulsion step. This corrects for the variable amount of water adsorbed on the harvested disks. Typically the Servall centrifuge is used for the second slow speed step, at 10,000 r.p.m. (10,000 × g) for 10–15 minutes, instead of the International No. 2 for 30 minutes. The virus pellet which sediments during ultracentrifugation is resuspended in a phosphate buffer of 0.01M instead of 0.1M.

EXPERIMENTAL RESULTS.—The compounds screened were purine analogs (Table 1) and naturally occurring purines (Table 2). The percentage of increase or decrease in TMV multiplications is based on the average virus yield of 3 independently treated samples as compared to the mean of 3 leaf disk samples floating on the solvent used (distilled water or neutralized 0.1 per cent sodium bicarbonate). On the basis of

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[2] The sulfate salt of this compound was tested.

TABLE 1.—*Percentage of decrease or increase in TMV multiplication in leaf disks floating on solutions of purine analogs*

Compound	Conc. of solution in mg./100 ml.	Percentage of Increase (+) Decrease (—) in TMV multiplication	Average percentage of deviation from mean	Effect of Compound on tissues[a]	Compound	Conc. of solution in mg./100 ml.	Percentage of Increase (+) Decrease (—) in TMV multiplication	Average percentage of deviation from mean	Effect of Compound on tissues[a]
8-azaguanine	1	0	1.7	0	2,6-diaminopurine sulfate	10	—90	8.6	0
8-azaguanine	10	—10	3.4	0	2,6-diaminopurine sulfate	10	—96	8.8	0
8-azaguanine	10	—24	3.9	0	2-oxy-6-aminopurine sulfate[b]	(Sat.)	—14	4.6	3
8-azaguanine	15	—16	0.2	0	2-oxy-6,8-diamino-purine sulfate	2	+12	2.7	0
8-azaguanine	15	— 9	9.0	1	2-oxy-6,8-diamino-purine sulfate	10	0	3.2	0
8-azaguanine	50	—56	6.6	2	2,6-diamino-8-hydroxypurine	7	—16	4.0	0
8-azaguanine	75	—60	7.7	5	2,6-diamino-8-azapurine	2.5	—19	2.3	0
8-azaadenine	1	—14	4.1	0	2,6-diamino-8-azapurine	10	—18	3.3	0
8-azaadenine	5	—71	5.0	0	2,6-diiodopurine	10	—10	0.8	0
8-azaadenine	7.5	—69	4.5	0	2,6-diiodopurine	(Sat.)	—17	0.9	0
8-azaadenine	10	—94	1.5	0	2,8-dichloro-6-hydroxypurine	10	—49	5.7	3
8-azaadenine	10	—83	6.5	0	2,6,8-trichloropurine	2	+ 6	1.9	0
8-azaadenine	10	—91	7.5	0	2,6,8-trichloropurine	10	+17	5.1	0
8-azaadenine	15	—88	6.6	0	2,6-dichloro-7-methylpurine	2	+ 6	3.8	3
8-azaadenine	15	Not assayed	...	5	8-chloroxanthine	10	—26	3.3	3
8-azahypoxanthine	10	+ 5	3.8	1	8-chloroxanthine	50	—44	4.6	5
8-azahypoxanthine	15	+ 2	3.2	1	2-thioxanthine	10	+10	1.3	0
8-azahypoxanthine	50	+19	0.6	0	2-thioxanthine	50	+22	3.0	0
8-azahypoxanthine	75	+ 5	3.1	2	Thioguanine	2.5	—10	3.2	0
8-azaxanthine	2.5	—16	0.7	0	Thioguanine	10	—20	4.5	0
8-azaxanthine	10	—18	3.7	0	2-chloroadenine	(Sat.)	— 9	11.5	4*
Benzimidazole	10	—26	1.4	0	2-chloroadenine	(Sat.)	— 3	5.8	4*
Benzimidazole	40	—49	4.8	3	2-methyladenine	9.5	—31	3.8	4
6-nitrobenzimidazole	10	—20	4.3	0	2-methylthioadenine	6	—21	2.4	0
2-methylbenz-imidazole	10	+12	2.5	0	2-methylthioadenine	12	—23	7.8	0
2-methylbenz-imidazole	50	—11	2.1	0	2-iodoadenine	10	—20	2.7	0
2,5-dimethylbenz-imidazole	10	—37	0.0	3	7-methyladenine	4.5	+10	3.1	0
2-ethyl-5-methyl-benzimidazole	2.5	— 2	3.6	0	7-methyladenine	9	+21	9.3	0
2-ethyl-5-methyl-benzimidazole	10	—25	0.8	0	2-chloro-6-amino-8-hydroxypurine	8.5	—10	4.6	0
2,5,6-trimethyl-benzimidazole	2.5	—15	4.8	0	2-chloro-6-amino-8-hydroxypurine	(Sat.)	— 7	4.4	0
2,5,6-trimethyl-benzimidazole	10	—34	4.8	5	2-chloro-7-methyl-adenine	10	— 6	4.4	0
2-azaadenine	1	— 9	1.6	0	2-chloro-9-methyl-adenine	7.5	—21	4.0	3
2-azaadenine	5	—34	3.9	0	2,8-dichloroadenine	8.5	—40	7.1	4*
2-azaadenine	5	—28	6.5	0	2,8-dichloroadenine	(Sat.)	—44	6.8	4*
2-azaadenine	10	—78	5.9	3					
2-azaadenine	10	—58	4.6	0					
2-azaadenine	15	—87	13.2	6					
2,6-diaminopurine sulfate	10	—84	6.1	0					
2,6-diaminopurine sulfate	10	—89	4.7	0					

[a] Key to description of effect of compound on tissues in Tables 1 and 2: 1—Slight chlorosis; 2—Very chlorotic, but turgid; 3—Watersoaking in minute spots on some, but not all, disks. Most of tissue area green and turgid; 4—Isolated necrotic spots. Most of tissue area green and turgid; 4*—Same as 4; in addition, disks were greener than controls; 5—Badly watersoaked, or chlorotic and flaccid.

[b] Reported occurring in croton bean as a riboside (1).

over 600 samples assayed, it was found that 95 per cent of the samples assayed did not differ from their respective means by more than 10 per cent.

Of the triazolo analogs of naturally occurring purines tested (Table 1), 8-azaadenine and 8-azaguanine were effective in inhibiting TMV multiplication; 8-azahypoxanthine and 8-azaxanthine caused no significant effect. Other purine analogs that significantly inhibited TMV multiplication with little or no observable injury to leaf disks are: benzimidazole, 6-nitrobenzimidazole, 2-ethyl-5-methylbenzimidazole, 2-azaadenine, 2,6-diaminopurine, thioguanine, 2-methylthio-

TABLE 2.—*Percentage of decrease or increase in TMV multiplication in leaf disks floating on solutions of naturally occurring purines*

Compound	Conc. of solution in mg./100 ml.	Percentage of Increase (+) Decrease (—) in TMV multiplication	Average percentage of deviation from mean	Effect of Compound on tissues[a]
Guanine	(Sat.)	+ 9	3.8	0
Guanine (HCl)	(Sat.)	+10	5.0	0
Adenine and Guanine (HCl)	10 (each compound)	+16	2.8	0
Adenine	10	+ 8	3.0	0
Adenine	10	+21	3.9	0
Hypoxanthine	10	— 2	2.1	1
Hypoxanthine	10	+ 5	3.2	0
Hypoxanthine	10	+ 5	2.5	0
Adenylic acid[b]	50	+24	3.8	0
Xanthine	20	0	1.1	0
Xanthine	20	0	2.8	0
Xanthine	(Sat.)	—14	2.9	2, 3
Caffeine	10	+11	6.3	0
Caffeine	50	— 2	2.5	1
Uric Acid	10	+22	2.0	0
Uric Acid	50	+26	1.5	0
Theobromine	10	+ 8	1.7	0
Theobromine	50	+ 8	1.0	0
Theophylline	10	+11	1.0	0
Theophylline	50	— 6	2.0	0

[a] Same key followed as in Table 1.
[b] Adenylic acid is a naturally occurring nucleotide.

adenine, and 2-iodoadenine. The analogs 2-thioxanthine and 7-methyladenine appear capable of slightly increasing virus formation.

Of the naturally occurring purines tested (Table 2), none greatly increased TMV multiplication. In 1 trial, adenine was significantly stimulatory, as was uric acid, and the naturally occurring nucleotide, adenylic acid.

In addition to the tabulated compounds, the following miscellaneous compounds were tested at the indicated concentrations (in mg./100 ml.) with little or no effect on TMV multiplication in leaf disks: imidazole, 10 and 50; imidazole dicarboxylic, 10 and 50; 2-methyl imidazoline bitartrate, 10 and 50; allantoin, 10 and 50; methyl pyrazolone, 10 and 50; caffeic acid, 10 and 40; trigonelline, 10 and 50; benzotriazole, 0.2 and 1; 2-mercaptobenzoxazole, 0.2 and 1; beta-methyl umbelliferum, 10 and saturated; scopoletin, 0.5 and saturated; thiohydantoin, 10 and 50; 1-acetyl-5-(3-thenyl-) 2-thiohydantoin, 10 and 50; N-(5-nitro-2-furfurylidene)-1-aminohydantoin, 10 and 50.

ACTION OF 8-AZAADENINE AND 2,6-DIAMINOPURINE.—Since 8-azaadenine and 2, 6-diaminopurine were the most effective inhibitors of TMV multiplication, the action of these purine analogs was studied further, with respect to the relation between concentration of analog and the percentage of inhibition of TMV multiplication, the relation of time to the inhibitory action of the analog, and the effect of various compounds on the inhibition of TMV multiplication by either analog. The experimental set up for these experiments was identical for both compounds, and the procedure the same as for the compounds screened (Tables 1 and 2). For brevity, the data will be presented for 2,6-diaminopurine; results obtained with 8-azaadenine are similar.

Relation of concentration of analog to inhibitory action.—A series of concentrations between 0 and 50 mg./100 ml. of 2,6-diaminopurine was supplied to leaf disks, and the relative virus yield (in optical density units) determined for each concentration (Fig. 1). A line was drawn through the calculated mean optical density points representing the mean virus yield for each concentration of compound supplied. The data indicate that there is a decrease in the amount of TMV formed with increasing concentrations of analog. The decreased virus yield with increasing concentration of analog forms 2 approximately straight lines. The slope of one line which is between 0 and 10 mg./100 ml. of 2,6-diaminopurine differs from the slope of the other line which is between 10 and 50 mg./100 ml. of 2,6-diaminopurine. A similar change in slope occurs with 8-azaadenine at approximately 5 mg./100 ml.

Relation of time to inhibitory action.—Collections of controls and leaf disks on 2,6-diaminopurine were made daily for 6 days to determine the relation of

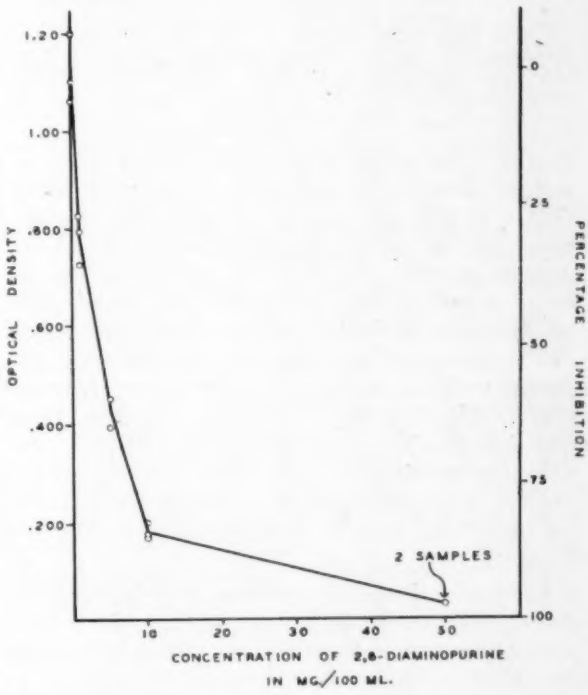

FIG. 1. Increase in percentage of inhibition of TMV multiplication with increasing concentrations of 2,6-diaminopurine supplied to leaf disks. Each point represents the optical density obtained for a single sample. The curve has been drawn through the calculated mean for each concentration.

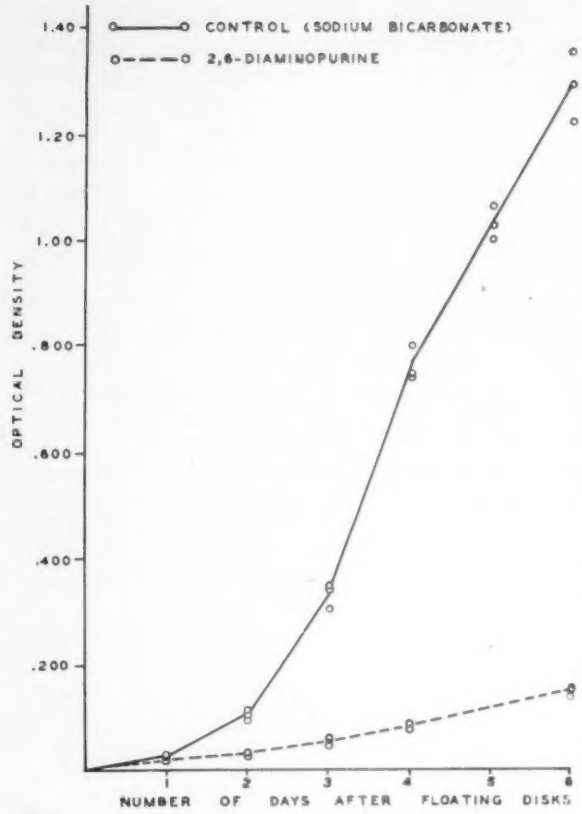

FIG. 2. Relation of time to the inhibitory action of 2,6-diaminopurine (10 mg./100 ml.) on TMV formation in tobacco leaf disks. Lines are drawn through the respective mean values.

time to the inhibitory action of the analog. Virus assays for these collections, plotted for the 6-day period (Fig. 2), indicate that 2,6-diaminopurine did not completely stop TMV multiplication throughout the disks at any time. The decreased slope caused by the chemical reflects the greatly reduced rate of TMV multiplication in the leaf disks on 2,6-diaminopurine. For the concentration tested (10 mg./100 ml.), 2,6-diaminopurine appeared to reach maximum effectiveness by the second day. Calculations based on these data show that the percentage inhibition of TMV multiplication remained essentially constant from the second day to the sixth day (81.3 per cent inhibition for the second day and 88.7 per cent inhibition for the sixth day).

Effect of various compounds on TMV inhibition by purine analogs.—To learn some of the possible relations between various purines and related compounds and TMV multiplication and to gain evidence as to whether 2,6-diaminopurine and 8-azaadenine inhibit TMV multiplication by inhibition of the utilization of some metabolite, compounds were tested for their ability to reverse the inhibition by either analog. In this type of experiment the inhibition caused by 2,6-diaminopurine or 8-azaadenine is compared with inhibition resulting from solutions of the same concentration of analog containing appropriate concentrations of a potential reversing agent (equivalent on a molar basis to the 10 mg./100 ml. of adenine capable of causing complete reversal of the inhibition by either analog). Results of a typical experiment with 2,6-diaminopurine (Table 3) show that adenine caused complete reversal of the inhibition by 2,6-diaminopurine. Reversal by both adenosine and adenylic acid was very high but not complete. Guanine hydrochloride demonstrated appreciable reversing activity in all experiments, but guanine was never so complete a reversing agent as adenine. In contrast to guanine, guanosine did not cause a significant reversal of TMV inhibition in any experiment. Guanylic acid also failed to reverse TMV inhibition in one test, and caused excessive water-soaking injury to the disks in another, so that no virus assays were made in the latter trial. The compounds xanthine, hypoxanthine, 4-amino-5-imidazole carboxamide, 4-formamino-5-imidazole carboxamide, and p-aminobenzoic acid demonstrated little or no reversing activity.

DISCUSSION.—Since the screened compounds were supplied to leaf disks at least 24 hours after inoculation with TMV, the effect of a compound on virus yield is probably due to an effect on multiplication, and not on the infection process (10). Two triazolo analogs, 8-azaadenine and 8-azaguanine, significantly inhibited TMV multiplication, but 2 other analogs, 8-azaxanthine and 8-azahypoxanthine, did not. If Matthews' local lesion results with the latter compound (4) represent an effect on virus multiplication, and not primarily an effect on lesion production, or infection, then it ap-

TABLE 3.—*The action of various purines and related compounds in reversing TMV inhibition by 2,6-diaminopurine*

Compound	Concentration (M)	Mean yield of TMV (optical density)	Percentage inhibition of TMV multiplication
Control ($NaHCO_3$)	.....	0.968	.....
2,6-diaminopurine	0.0005	0.221	—77.4
Adenine[a]	0.0007	0.948	— 2.1
Adenosine[a]	0.0007	0.743	—23.2
Adenylic acid[a]	0.0007	0.806	—16.7
Guanine[a]	Saturated	0.676	—30.2
Guanosine[a]	0.0007	0.277	—71.4
Guanylic acid[a]	0.0007	0.358	—63.0
Xanthine[a]	0.0007	0.389	—60.0
4-amino-5-imidazole carboxamide[b]	0.0005	0.261	—73.0
4-formamino-5-imidazole carboxamide[b]	0.0005	0.264	—72.7
Hypoxanthine[a]	0.0007	0.313	—67.7
p-aminobenzoic acid[a]	0.0007	0.278	—71.3

[a] In addition, each solution contained 2,6-diaminopurine (0.0005M).

[b] Tested only with 2,6-diaminopurine, and not with 8-azaadenine.

pears that 8-azahypoxanthine inhibits the formation of lucerne mosaic virus, but not TMV.

Three compounds that caused appreciable inhibition of TMV multiplication in our work, 8-azaadenine, 2-azaadenine, and 2,6-diaminopurine, are structurally related to the normally occurring purine, adenine. The specificity of structure required in the inhibition process is indicated by the large number of other compounds tested that resemble the effective analogs and adenine, but are relatively or completely inactive. Differences in cell permeability to various compounds may also be partly responsible for the differences in activity of these compounds.

In general, naturally occurring purines had little effect on TMV multiplication in tobacco leaf disks. This suggests that under the conditions employed, the naturally occurring purines tested do not limit TMV formation. However, in the presence of 2,6-diaminopurine or 8-azaadenine, adenine and guanine, as well as adenosine and adenylic acid, do limit virus formation, as is shown by their reversal of TMV inhibition.

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THE INFLUENCE OF TEMPERATURE ON THE DEVELOPMENT OF POWDERY MILDEW ON SPRING WHEATS [1]

M. C. Futrell and J. G. Dickson

SUMMARY

The development of powdery mildew incited by *E. graminis tritici* was studied on 15 susceptible, intermediate, or resistant spring wheat varieties grown in the greenhouse at 16°, 20°, 24° and 28°C. These 15 varieties showed a wide range in disease response when grown at the 3 lower temperatures. All susceptible varieties showed heavy infection on the leaf sheath and the leaf blades at 16°, 20°, and 24°C. In the resistant Sturgeon variety the leaf sheath was more susceptible than the leaf blade, reaching the maximum infection on the leaf sheath at 20°. The resistant Egypt Na 101 variety showed a low percentage of type 4 infection, reaching a maximum on the leaf blades at 24°. Powdery mildew showed a wider range of response to temperature on the resistant Hope variety than on the other varieties. The leaf blades of Hope were resistant at 16° and 20° but were moderately susceptible at 24°. The development of mycelium on the leaf sheaths of Hope plants was sparse at 16° and 20° whereas it was appreciably greater at 24°. Khapli, *T. timopheevi* and 2 common wheat selections, C. I. 12632 and C. I. 12633, from the cross (Illinois No. 1 × Chinese)[3] × *T. timopheevi* showed good resistance at all temperatures.

Powdery mildew incited by *Eyrsiphe graminis tritici* El. Marchal has caused serious losses in some of the soft red winter and hard red spring wheats during the past several years. Wheat varieties derived from Hope especially have been infected severely in the southeastern soft red winter and the southern section of the hard red spring wheat areas, especially in the seasons with above normal temperatures. Whereas new races of the pathogen have been identified (6, 9), field records and preliminary investigations in the greenhouse at Madison indicated that temperature was an important factor. Inoculation of Hope wheat with these new races of the pathogen showed a resistant reaction at 16 and 20° and susceptible reaction at 24°C. These results indicated the need for more detailed information on the influence of temperature upon the powdery mildew reaction of the wheats used in wheat improvement in these areas.

Investigations on the influence of temperature on the development of *Erysiphe graminis tritici* El. Marchal on wheat are limited, Pratt (8) showed that 20°C. was optimum for the development of powdery mildew

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on seedling leaves of Marquis wheat, and that no infection occurred at 30°C. Honecker (5) found that the powdery mildew fungus developed best on barley between 15° and 20°, and Corneli (3) showed that 18° to 20° favored maximum infection of barley seedlings by this fungus. Cherewick (2) obtained good infection on barley at 18° with less infection at 26°. He found that alternating barley plants between these 2 temperatures gave higher percentages of infection than was obtained at either constant temperature.

MATERIALS AND METHODS.—The development of *E. graminis tritici* race 5 (7) on the leaves of young plants of 15 varieties of wheat at 16°, 20°, 24° and 28°C. was studied in the greenhouse during the winter months in 3 years from 1949 to 1951. Resistant, intermediate, and susceptible varieties as determined by previous field tests[2], were used. The susceptible varieties included Henry (C. I. 12265)[3], Mida (C. I. 12008), Rescue (C. I. 12435), Kenya (R. L. 1373)[4], and Thatcher (C. I. 10003). Intermediate varieties used were Frontana (C. I. 12470) and Lee (C. I. 12488). The varieties that had shown field resistance were Hope (C. I. 8178), Khapli (C. I. 4013), Egypt Na 101 (C. I. 12100), Sturgeon (C. I. 11703), Progress (C. I. 6902), *Triticum timopheevi* Zhuk. and 2 selections from the cross (Illinois No. 1 × Chinese)[3] × *T. timopheevi* (1) designated as C. I. 12632 and C. I. 12633. Plants from the above varieties were grown to the 2-leaf stage in a greenhouse free of mildew; they were inoculated by dusting conidia from infected plants on the seedlings, and placed at the 4 temperatures. Notes were taken on the powdery mildew response of the leaf blade and leaf sheath in the manner described by Newton and Cherewick (7). The percentage of leaf blade and sheath area covered by the fungus stromata also were recorded.

The effect of temperature on ascospore maturation, discharge, and infection on leaves of Marquis wheat was studied during the winter of 1949-50. Dried wheat leaves containing perithecia with undifferentiated ascospores were clipped to moistened filter paper which was fastened to the bottom of Petri dishes and inverted over lantern globes. Plants of Marquis (C. I. 3641) grown to the 2-leaf stage in 4-in. pots were placed in this type of moisture chamber at 16°, 20°, 24° and 28°C. and a Petri dish with ascosporic inoculum was inverted over the top. Notes were taken on the number of days required for visible stromata to develop at each temperature. A similar set of leaves in Petri dishes were inverted over glass slides to determine the time of ascospore maturation and discharge at the 4 temperatures. Ascospore development was followed histologically in material fixed at given intervals.

RESPONSE OF VARIETIES.— A susceptible, type 4 (0-4 scale) response with a large part of the leaf blade and leaf sheath covered with mycelium was the general pattern shown by all susceptible varieties as illustrated in Figure 1A. The incubation period, on susceptible hosts, was shorter at 20° than at 16°. Under the short days and low light intensity of December and January, plants of susceptible varieties were killed by the fungus in about 4 weeks after inoculation at 16° and 20°. At 16° moderate to heavy conidial sporulation of the fungus occurred 2 weeks after inoculation, followed by a sparse development of perithecia about 2 weeks later. Abundant production of conidia and perithecia occurred at 20°. At 24° the mycelium was sparse with heavy production of conidia and no production of perithecia, and at 28° neither mycelium nor spores developed.

When resistant and intermediate varieties were infected at 16° and 20°, heavy perithecial production occurred within 3 or 4 weeks after inoculation. The intermediate varieties, Lee and Frontana, responded differently in the various tests conducted at different times during the spring and winter. Lee gave a susceptible response during the winter months and a type 2 reaction in spring tests. The seedlings and young plants of Frontana showed a range of response, but the exact pattern of infection was not correlated with either season or temperature. The interaction of light intensity, day length, and temperature on this variety was not studied. All susceptible varieties followed the general pattern of disease development shown for Henry, Figure 1A. Maximum disease development on both leaf sheath and leaf blade occurred at 20°, although there was very little difference in the percentage of leaf area covered in this variety at 16°, 20° and 24°. At 28° the susceptible varieties showed little or no disease.

Varieties and selections resistant in the field varied widely in powdery mildew reaction at the 4 temperatures in the greenhouse. The powdery mildew reaction of Hope showed a greater range in response to temperature than did other varieties. Sparse mycelial development of type 3 reaction appeared on the leaf sheaths of Hope at 16° and 20° while the most abundant mycelial growth of type 4 reaction occurred at 24° as shown in Figures 1B and 2A. The leaf blades of Hope plants showed flecking only at 16° and 20° and were moderately susceptible with type 4 mycelial development at 24°. There was sparse development of the fungus on the leaf blades and sheaths at 28°. The leaf blades of Hope growing at 16° and 20° were reinoculated with conidia collected from diseased plants growing at 24° to demonstrate that new biotypes had not been screened out at 24° and again no disease developed at the lower temperatures. With the same race present Hope showed good resistance under field conditions.

Progress and Sturgeon gave similar responses to powdery mildew. Limited stromata, type 4 reaction, developed on the leaf blades of these 2 varieties at the 3 lower temperatures. Mycelium and conidia

[2] Shands, R. G. Unpublished data, University of Wisconsin, Madison 6, Wisconsin.

[3] Accession number of the Section of Cereal Crops and Diseases, U. S. Department of Agriculture.

[4] Rust Laboratory number of Dominion Laboratory of Plant Pathology, Winnipeg, Canada.

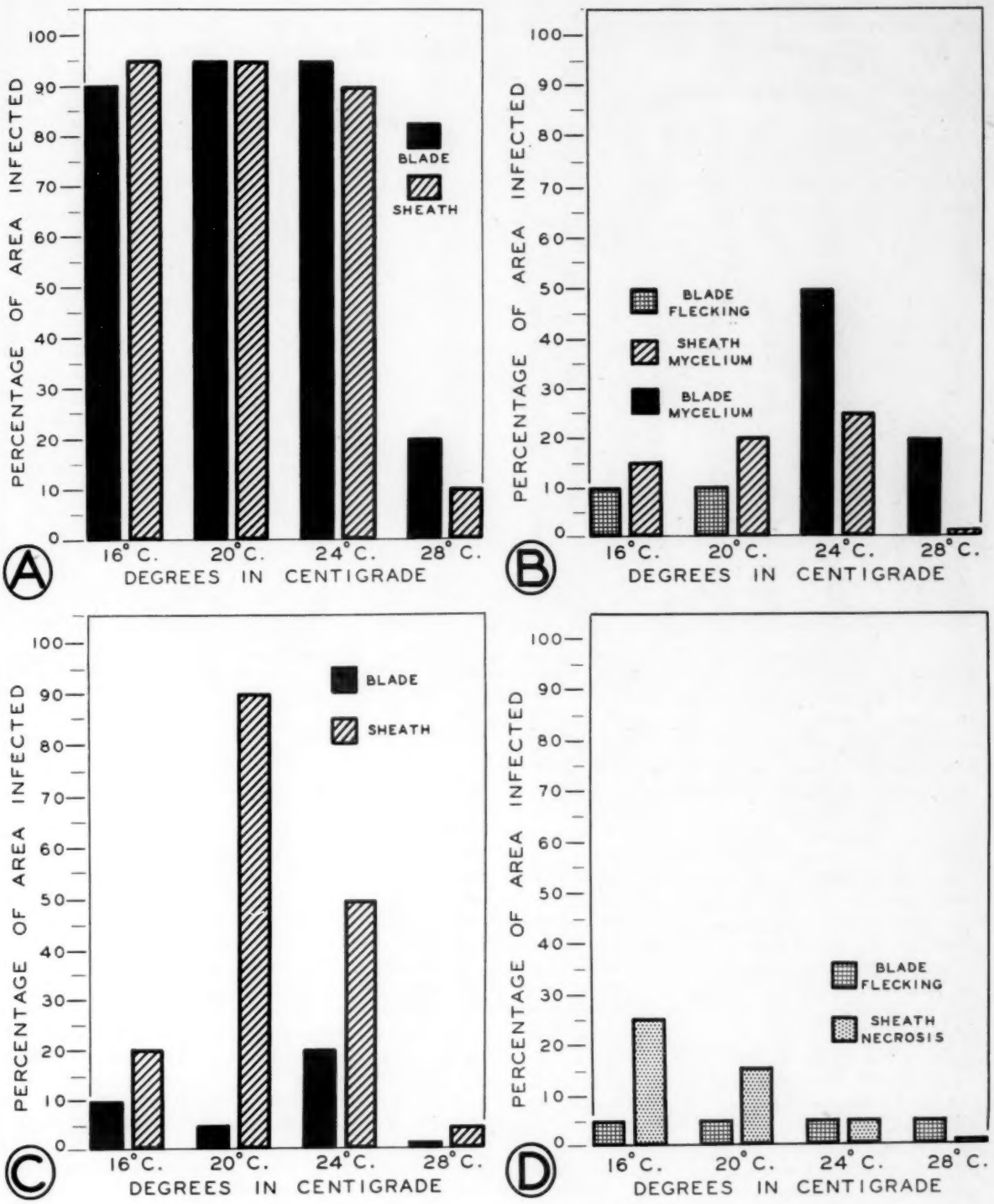

FIG. 1. Graphic presentation of the influence of temperature on powdery mildew reaction of four wheats grown in the greenhouse. A) Percentage area covered with Type 4 infection on Henry, susceptible. B) Percentage area affected by flecking and mycelium on Hope, resistant. C) Percentage area covered with Type 4 reaction on Sturgeon resistant under field conditions. D) Percentage leaf blade flecking and sheath necrosis on C. I. 12633, (Illinois No. 1 × Chinese)[3] × *T. timopheevi*, resistant.

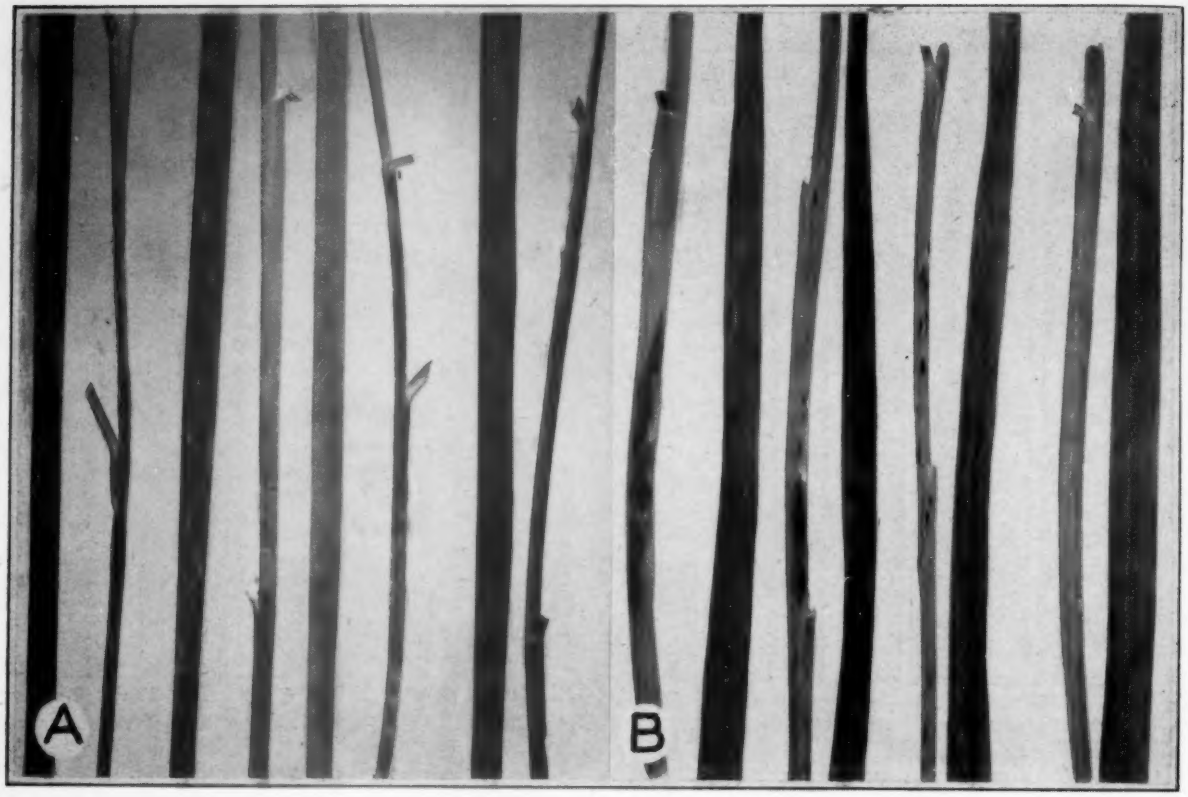

FIG. 2. The characteristic powdery mildew development and response of leaf sheath and blade of 2 resistant wheats grown in the greenhouse at 4 temperatures. A) Hope, leaf blade and sheath, left to right, grown at 16, 20, 24, 28°C. B) C. I. 12633, sheath necrosis and blade flecking, left to right, grown at 16, 20, 24, 28°C.

developed profusely on the leaf sheath of these varieties at 20° and 24° as illustrated for Sturgeon in Figure 1C. Sturgeon, a selection from a cross of Progress with Marquis, has been grown successfully for many years in the vicinity of Sturgeon Bay, Wisconsin, under powdery mildew epiphytotics.

A small number of type 4 stromata developed at all temperatures on the blades and sheaths of Egypt Na 101. The number was greater on the leaf sheath of plants at 16°, and decreased gradually with the rise in temperature. More powdery mildew developed on the leaf blades of Egypt Na 101, as in Hope, at 24° than at the lower temperatures. Perithecia developed in the mycelium on both the leaf blades and sheaths of Egypt Na 101 at the lower temperatures.

Khapli and *T. timopheevi* showed high resistance, and the 2 selections from a *T. timopheevi* cross, C. I. 12632 and C. I. 12633, were the most resistant of the common wheats tested. Under greenhouse conditions these 2 selections showed about 5 per cent flecking which was not influenced by temperature, (Figure 1D). When large numbers of conidia were brushed or dusted on the sheath tissue of these selections, large brown necrotic areas developed about a week after inoculation (Figure 2B). The brown sheath necrosis was most pronounced at the low temperatures and absent at 28° as shown in Figures 1D and 2B. The histological explanation of these necrotic areas has been given in detail elsewhere (4). These 2 selections have been nearly immune from the fungus in field tests at Madison.

INFECTION WITH ASCOSPORES.—Under greenhouse conditions the optimum temperature for ascospore infection on seedling plants of Marquis wheat was 20° where the first mycelium developed in 8 days. The most rapid discharge of ascospores occurred at 24° and 28°; however, the fungus failed to establish infection at 28°. Ascospores matured and liberated at 28° developed small, weak germ tubes. A longer period of time was required for liberation of ascospores at lower temperatures, but the ascospores were very large and germinated vigorously. Failure of the fungus to produce good ascosporic inoculum, coupled with weak spore germination, probably accounted for the failure of the ascospores to incite infection at 28°. The upper temperature limits of *E. graminis tritici* for disease production on the wheats tested appeared to be near 28° under greenhouse conditions during the winter months (Table 1).

DISCUSSION.—As shown earlier by Pratt (8) the optimum temperature for powdery mildew infection on seedling plants of Marquis wheat was near 20° when inoculated with conidia. However, infection and disease development at 16° and 24° was only slightly

TABLE 1.—*The time required for ascospore liberation and mycelial development by E. graminis tritici on Marquis wheat in the greenhouse at 4 temperatures*

Temperature	Time required for	
	Ascospore discharge	Visible mycelium on leaves
°C.	hours	days
16	112	11
20	96	8
24	72	9
28	72	[a]

[a] No infection occurred at 28°C.

less than at 20° on the susceptible varieties used in the present investigation. At 16° the plants remained vigorous which allowed sporulation over a long period of time as pointed out by Cherewick (2).

Hope wheat was moderately susceptible at 24°, which suggests an explanation in part for the recent prevalence of powdery mildew on Hope and its derivatives under the warm climate of the Southeastern United States (6, 9). Field resistant varieties differed in their response to powdery mildew at various greenhouse temperatures. Hope showed more resistance at 16° with both leaf blade and sheath increasing in susceptibility at 24°. Sturgeon showed the greatest susceptibility of leaf sheath at 20°. The sheath necrosis of C. I. 12633 was most pronounced at 16° and was absent at 28°. Varietal reaction indicated that environment influenced the expression of resistance differently in the several varieties tested. The reaction of varieties such as Frontana suggested that light intensity, day length, and temperature all influence powdery mildew development. Frontana is classified as an intermediate spring type and, in this respect, is different from other varieties studied.

The absence of sheath necrosis on C. I. 12633 at 28° indicated that the fungus probably never completely penetrated the epidermal cell walls since histological examination (4) of this resistant selection showed necrosis only when the fungus contacted the host cytoplasm.

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ROOT AND CROWN ROTS OF RED CLOVER IN WISCONSIN AND THE RELATIVE PREVALENCE OF ASSOCIATED FUNGI [1]

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SUMMARY

Surveys were made in 1949, 1950, and 1951 to determine the prevalence and severity of root and crown rots of red clover in Wisconsin and to ascertain the kinds and relative prevalence of the fungi associated with this disease complex on post-seedling plants.

The severity of the rots varied greatly from year to year, being most severe in 1950 and least severe in 1951. Plants of all ages were infected but the rots were more severe in second-year plants than in first-year plants and within each of these groups the severity increased from the beginning to the end of the growing season. Stands decreased as the severity of the rots increased. Tap roots, secondary roots, and frequently the crowns were diseased. Both cortical and vascular tissues were affected but not always simultaneously; sometimes the infection was mostly cortical and sometimes it was mostly vascular.

Fusarium oxysporum, F. solani, F. roseum, Phoma spp., *Rhizoctonia* spp., and *Gliocladium roseum* were among the most common fungi isolated from diseased roots. The relative prevalence of these species varied with locality, age of plants, season of the year, cultural practices, and other factors. *Fusarium oxysporum* was the most prevalent fungus isolated. It was particularly abundant in first-year plants. *F. solani* and *F. roseum* were the next most common species. These were most abundant in roots of second-year plants. All these fungi were present in samples from all parts of the state but *F. solani* was less prevalent in plants grown on Plainfield sand than in those grown on other types of soil. The microflora of the various parts of the root system were also different. *Pythium* spp. were isolated only from cortical tissues while some of the other fungi were most frequently isolated from vascular tissue.

One of the most important problems in the production of red clover (*Trifolium pratense* L.) is the establishment and maintenance of stands. Several factors are involved, one of which is the root and crown disease complex. Stand losses are most conspicuous in the second year, but also occur commonly in the first. In the humid areas of the United States, few red clover fields remain productive beyond the second year after seeding. Occasionally fields are plowed in the summer of the second year because the plants fail to recover after the first hay crop has been removed. Most red clover fields yield 2 crops of hay, or 1 crop of hay and 1 crop of seed, and then are abandoned because third year stands are too poor to produce profitable crops.

This problem has been apparent for many years. Pieters (23) and Fergus (7) stated that red clover failures have occurred in the United States since 1900. Many others (1, 8, 16, 18, 27, 34) also have called attention to the problem and have reported on the various types of root and crown rots and their effects on stand. Among the fungi listed as incitants have been *Sclerotinia trifoliorum* Eriks. (3, 10, 25, 35), *Fusarium* spp. (1, 3, 6, 8, 14, 16, 18, 21, 28, 29, 36, 37), *Rhizoctonia* spp. (22, 25, 34), *Colletotrichum trifolii* Bain & Essary (7, 20), *Sclerotium bataticola* Taub. (13), *Thielaviopsis basicola* (Berk. & Br.) Ferr. (25), *Cylindrocarpon ehrenbergi* Wr. (4), *Plenodomus meliloti* Dearn. & Sanf. (26), *Leptodiscus terrestris* Gerdemann (9), *Pythium* spp. (2, 11), and an unidentified low temperature Basidiomycete (5). Other fungi also have been isolated from roots and crowns of red clover (15, 17).

The objectives of the present investigation were: 1) to determine the kinds of root and crown rots of red clover in Wisconsin, 2) to obtain further information concerning the effects of these rots on stand and vigor, and 3) to determine the kind and relative prevalence of the fungi associated with diseased roots, including an analysis of the effects of stage of development of the plants, season of the year, locality in which the plants were grown, and other factors on the microflora.

MATERIALS AND METHODS.—The observations reported here were made in 1949, 1950, and 1951. In 1949, most of the plants studied were obtained from fields in the vicinity of Madison. In 1950 and 1951, they were collected from representative fields located on 6 typical soil types of the state. These soil types were: Almena silt loam, Longrie silt loam, Miami silt loam, Omega loamy sand, Ontonagon silty clay loam, and Plainfield sand located near Marshfield, Sturgeon Bay, Madison, Spooner, Ashland, and Hancock, respectively. Samples were taken at regular intervals throughout the summer and fall months from at least 2 fields at each location, at least one of which was a first-year and another a second-year stand. Each sample consisted of 200 or more plants collected at random.

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TABLE 1.—*Losses in stand during the first 18 weeks after seeding of 10 strains of red clover grown in the field at Madison, Wisconsin in 1949*

Strain	Stand in percent[a]		Losses in stand in percent		
	5 weeks after seeding	18 weeks after seeding	First 5 weeks	6th through 18th week[b]	Total
Dollard	64	39	36	39	61
Ottawa	64	35	36	46	65
Kenland	63	35	37	45	65
Midland	60	35	40	41	65
Cumberland	61	32	39	47	68
Wegener	55	31	45	44	69
Rahn	54	31	46	44	69
Wisconsin Mildew Resistant	55	30	45	46	70
Emerson	58	28	42	52	72
Common Mammoth	46	26	54	44	74
Average	58	32	42	45	68

[a] Stands based on percentage of viable seed sown.
[b] Stands at beginning of 6th week were considered 100 per cent stands in determining losses for this period.

More than 48,000 plants ranging in age from 5 weeks to 19 months were lifted, washed, and critically examined during the course of the investigation to ascertain symptoms and extent of injury to the roots. The plants in each sample were classified into 7 classes according to the amount of root decay. These classes and the values assigned to them were: healthy = 0, trace = 5, slight = 20, moderate = 40, moderately severe = 60, severe = 80, and very severe = 95. After classification, the number of plants in each class was multiplied by the class value, the sum of the products determined and the latter divided by the total number of plants in all classes to give the average disease severity rating for the sample.

All microflora determinations were based on plating studies. The number of root sections plated was 3000 in 1949, 7500 in 1950, and 6700 in 1951. Approximately half of the sections plated each year was from first-year plants; the other half was from second-year plants. At the beginning of the isolation work, it was noted that the relative prevalence of the different fungi associated with diseased roots was not always identical for all parts of the root system. Consequently, sections from primary and secondary roots were plated separately. In addition, separate isolations were made from the cortex and the vascular cylinder. All sections plated were taken, insofar as possible, from the margins of diseased and healthy tissue.

Surface sterilization was with a 1:1 solution of 50 per cent alcohol and 1 per cent calcium hypochlorite. The sections were submerged in the solution from 1 to 3 minutes depending on the size of the section. Rinsing after treatment had little effect on results and therefore was omitted.

Plating was done in Petri plates on potato-cerelose-agar (24) and the sections were incubated at room temperature. As soon as colonies appeared, a few hyphal tips from each were transferred to potato-cerelose-agar slants for later identification. The Snyder and Hansen system (31, 32, 33) was followed for the nomenclature of the fusaria.

As an additional source of material and in order to facilitate continuous observation of disease development, supplementary plots were sown at Madison April 26, 1949 and June 1, 1950. In 1949, 10 strains (Table 1) were seeded at the rate of 1200 viable seeds per row, in rows 18 ft. long and 1 ft. apart, without a nurse crop. This nursery included 80 rows, there being 2-row plots of each strain replicated 4 times. One of the paired rows of each strain in each replicate was used for root samples and the other was left undisturbed for making stand counts and observing plant development. In 1950, 3 strains, Dollard, Kenland, and Wisconsin Mildew Resistant, were used. These were sown broadcast in 3 ft. × 8 ft. plots, replicated 12 times. Six of the replicates were used for root samples while the others were used for stand determinations and for making general observations. This nursery was sown with oats as a nurse crop. In both years the nursery plots were arranged in a randomized block design and all seed lots were inoculated with "Nitragin", a commercial preparation of *Rhizobium trifolii* Frank.

The field site selected for these plots was located on the Wisconsin Agricultural Experiment Station West Hill Farm, at Madison, Wisconsin. Red clover had been grown continuously on the area for several years and the soil was known to be infested with root rot fungi. The soil type was a Miami silt loam. The 2 nurseries seeded on this site are referred to as Madison-A plots to distinguish them from the more representative fields also located at Madison which are designated as Madison plots. The latter were considered more representative because they had previously had a corn-oats-legume rotation. Most of the data given for 1949 were obtained from the 1949 Madison-A nursery.

Stand losses were determined only on the Madison-A plots. This was done not for the purpose of estimating stand losses for the entire state but to gain some estimate of the extent of the losses that might occur under conditions favoring root rot development, and also to measure some of the effects of these rots on stand. In

1949, stand counts were made June 1 and August 31, which was 5 weeks and 18 weeks after seeding, respectively. In 1950, the counts were made June 30, July 29, September 3, and October 20. Additional counts were made in both nurseries during April of the second year to determine losses during the winter months.

EXPERIMENTAL RESULTS.—*Kinds of rots and symptoms.*—Some of the most common kinds of rots observed in this survey are illustrated in Fig. 1. Plants of all ages were infected. Crowns, tap roots, and secondary roots showed decay. Both cortical and vascular tissues were diseased but not always simultaneously. For example, some tap roots had mostly vascular discoloration (Fig. 1, D) while in others (Fig. 1, E) the rotting was entirely cortical. Fig. 1, C illustrates a very common type of damage to the secondary roots. Numerous local infections scattered throughout the root system not only checked the further development of affected root tips but frequently cut off entire branches thus reducing the effective root system. In the early stages of development, these infections advanced mostly in the cortical areas. Later, they frequently extended through the entire diameter of the root. Fergus and Valleau (8) reported that primary injury appeared to be limited to the cortical region but usually there was a decayed appearance throughout. They stated that damage in the smaller roots apparently extended beyond the cortex.

Vascular discoloration (Fig. 1, D) was first observed in field-grown plants 2–3 months old. It frequently was found before there were conspicuous lesions on the external parts of the tap roots. The extent and intensity of the vascular discoloration varied greatly. Many roots showed no discoloration whatsoever whereas in others the discoloration extended from the tip of the primary root to the crown. Whether or not all of the vascular discoloration in the tap roots of red clover is caused by fungi has not been established. Other factors also may be able to bring about this condition.

Apparently fungi can gain entrance to the roots in several ways. Young (36) noted that entrance is attained through broken secondary roots and through insect wounds in the crowns. Kreitlow and Hanson (18) reported that fungi enter the roots through wounds in the crown and tap root. Cortical ruptures produced by secondary roots are possible avenues of entrance. Fig. 1, D shows continuity between the decayed areas of the stems and the vascular core of the tap root suggesting that entrance may have been gained through diseased stems. It is likely that fungi gain entrance to clover roots in all these ways.

Insect injury (Fig. 1, F) was found on both first- and second-year plants. Sometimes it was external; sometimes internal. Fresh insect wounds on the roots were free from discoloration at first but quickly became infected and discolored. There is no doubt that insects attacking clover roots facilitate the development of root rots. In areas where clover root borers were prevalent, root rots were very severe.

Severity of rots.—Annual surveys (12) have shown that root and crown rots are perennially prevalent throughout Wisconsin. Their severity varies greatly from year to year and locality to locality, depending on the amount of winter injury, insect populations, rainfall, and other factors. Differences between fields in the same locality are also sometimes great. This was illustrated at Madison in both 1949 and 1950 when plants on land previously cropped to red clover for several consecutive years had more than twice as much rot as plants grown in fields less than a mile away where crop rotation had been practiced. All these fields had the same strains of red clover and were on the same type of soil.

The average severities for all fields sampled in Wisconsin during the growing seasons of 1950 and 1951 are given in Fig. 2. Similar data were obtained in 1949. First-year stands had much less root rot than second-year stands and generally all stands showed a gradual increase in severity from June to October. Cool, wet weather during the growing season favored the production of new roots and prolonged life of diseased plants but recovery was rarely sufficient to enable more than a small percentage of the plants to survive a second Wisconsin winter.

On the continuous clover plots, the average severity for first-year stands in 1949 ranged from 48 on June 25 to 72 on October 27. In 1950, it ranged from 32 on June 30 to 50 on October 20. Whereas the average severity for these plots was much greater than the average severity for farmers' fields (Fig. 2), it followed a similar trend, increasing as the season advanced. Second-year stands in both these nurseries were so poor that the plots were abandoned. Surviving plants in both nurseries showed severe root and crown rotting.

Losses in stand.—The heavy losses in stand for the 1949 Madison-A plots are shown in Table 1. For the first 5 weeks after seeding, the percentages of plants lost ranged from 36 to 54 with an average loss of 42 per cent. From the 6th through the 18th week, losses

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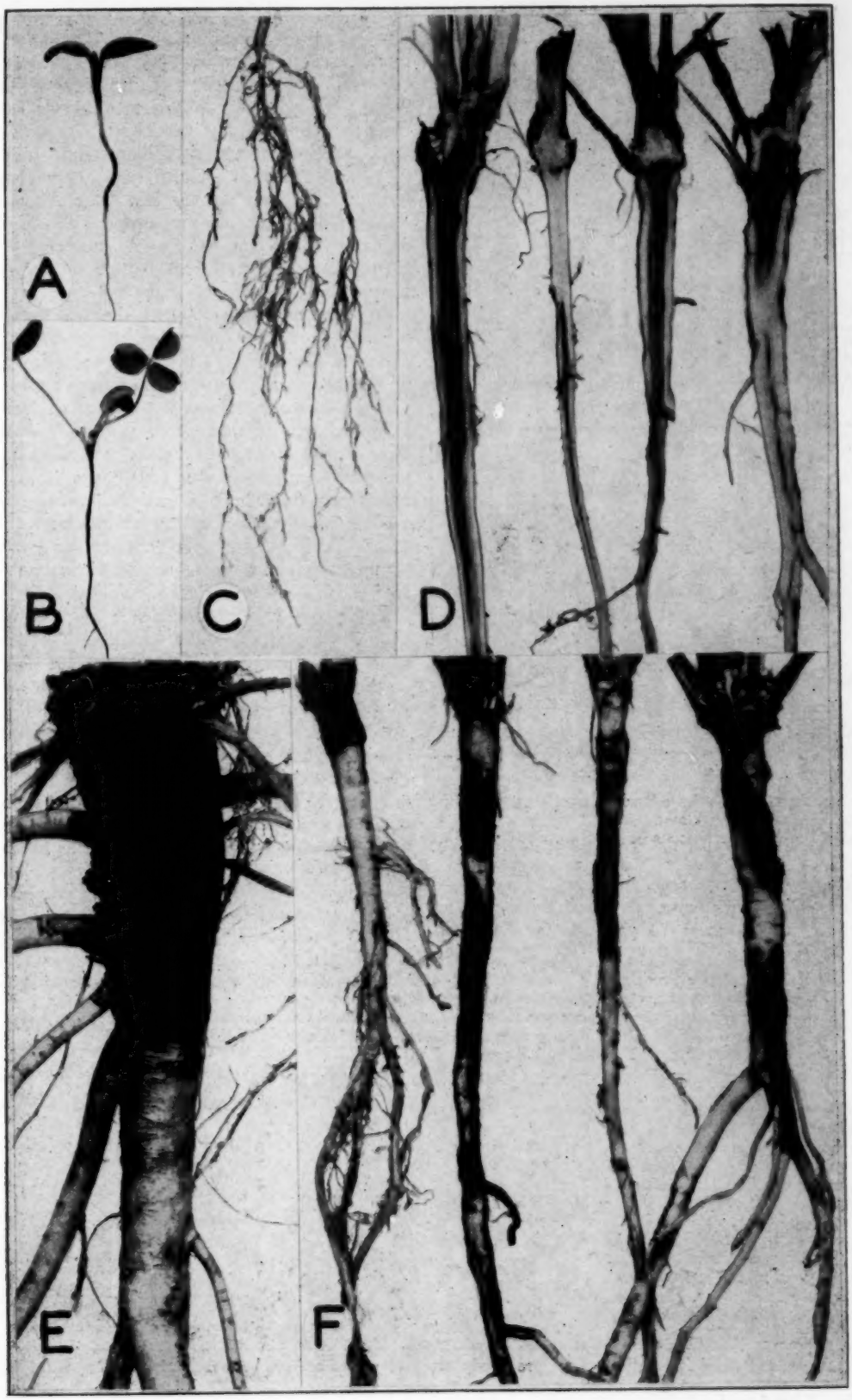
FIG. 1. Symptoms of root and crown rots of red clover in Wisconsin. A) Seedling with cortical discoloration of the upper part of the tap root and lower part of the hypocotyl. B) Three weeks old plant showing severe decay of the tap root and lower part of the crown; cortical areas were completely destroyed and vascular core was discolored. C) Secondary roots of a 6 weeks old plant showing numerous local infections. D) Longitudinal sections of tap roots and crowns of plants 6 months old showing vascular discoloration; the discoloration in 3 of the roots was continuous with the decay in diseased stems. E) Cortical rot of the upper part of the tap root of a second-year plant; vascular core was healthy. F) Insect injury and associated rot in the external areas of the tap roots of plants 4½ months old; root on left was not injured.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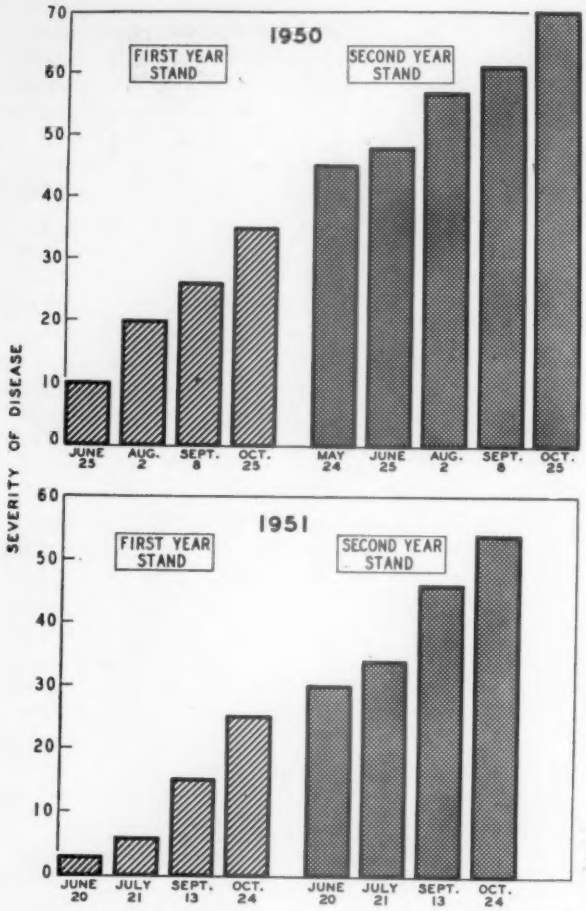

FIG. 2. Average severity in Wisconsin of root and crown rots in first- and second-year stands of red clover at various times during the growing seasons of 1950 and 1951.

ranged from 39 to 52 per cent, averaging 45 per cent. The total loss in stand for the 18 weeks ranged from 61 per cent for Dollard to 74 per cent for Common Mammoth. Only 1.2 per cent of the plants were still alive on April 25, 1950. Similar data were obtained in the nursery seeded in 1950. The average loss for the first 4 weeks after seeding was 68 per cent[3]. Average losses for subsequent periods were: 5th through 8th week, 30 per cent[4]; 9th through 14th week, 16 per cent; 15th through 20th week, 16 per cent; and 21st through 47th week, 19 per cent. The total loss in stand for the first 47 weeks was 87 per cent. Losses of this magnitude do not commonly occur in Wisconsin but they demonstrate what can happen in areas where root and crown rots are severe. Fergus and Valleau (8) have reported that environmental factors determine whether or not root rots become a critical factor in the production of red clover in Kentucky. They stated that summer losses in Kentucky were greater than winter losses and suggested that stand failures were directly related to root losses caused by pathogens, and were most serious when environmental conditions were unfavorable for host development. Smith (30) made plant counts in a first-year stand in Wisconsin in November 1947 and again 6 months later and 12 months later. He found that 60 per cent of the plants failed to survive the first winter and that 59 per cent of the surviving plants died during the following 6 months.

Kinds and relative prevalence of fungi associated with diseased roots.—The kinds and relative prevalence of the fungi isolated from first-year stands grown on the Madison-A plots in 1949 are indicated in Table 2. *Fusarium oxysporum* was the most prevalent, comprising on the average 61 per cent of the total isolates. No other fungus comprised more than 9 per cent. In 1950 and 1951, samples were obtained from both first- and second-year stands grown at 6 locations in the state, as well as from the Madison-A plots. Data from the latter were similar to those obtained in 1949. They

[3] Based on 54,000 viable seeds sown.

[4] Stands at beginning of each period except the first were considered 100 per cent stands in determining losses for that period.

TABLE 2.—*Kinds and relative prevalence of fungi isolated on 4 different dates during the growing season of 1949 from the roots of first-year red clover plants grown at Madison, Wisconsin* [a]

Fungus	Percentage of total isolates				
	June 25	July 30	Sept. 3	Oct. 27	Ave.
Fusarium oxysporum	72	73	50	50	61
Gliocladium roseum	4	8	11	13	9
Rhizoctonia spp.	3	5	10	13	8
Phoma spp.	2	6	6	2	4
Fusarium roseum	6	1	3	7	4
Fusarium solani	2	2	4	3	3
Pythium spp.	4	2	2	1	2
Trichoderma spp.	5	1	2	T[b]	2
Mucor spp.	T	1	1	3	1
Fusarium moniliforme	1	1	2	1	1
Penicillium spp.	T	T	1	1	1
Alternaria spp.	T	T	T	2	1
Aspergillus spp.	T	T	T	T	T
Others	1	1	8	4	3

[a] Clover was seeded April 26, 1949 on land previously cropped to red clover for several consecutive years (Madison-A plots).

[b] Less than 0.5 per cent.

TABLE 3.—*Kinds and relative prevalence of the fungi isolated from first- and second-year red clover plants grown at 6 locations in Wisconsin in 1950 and 1951*

Fungus	Percentage of total isolates[a] First year stands 1950	First year stands 1951	Second year stands 1950	Second year stands 1951
Fusarium oxysporum	38	39	27	33
Fusarium solani	17	14	23	26
Fusarium roseum	9	11	17	13
Phoma spp.[b]	2	8	3	8
Rhizoctonia spp.	4	1	5	2
Gliocladium roseum	2	4	1	2
Alternaria spp.	2	4	4	3
Mucor spp.	2	1	4	1
Penicillium spp.	1	3	3	2
Aspergillus spp.	1	2	3	1
Chaetomium spp.	T[c]	1	1	2
Fusarium moniliforme	1	1	1	1
Pythium spp.	1	1	T	T
Trichoderma spp.	T	T	1	1
Colletotrichum destructivum	T	T	T	T
Miscellaneous[d]	3	2	3	3
Unidentified	17	8	4	2

[a] Average for all samples from all locations.
[b] No attempt was made in this survey to identify every isolate to species. When the isolates have been tested for pathogenicity, those of most importance will be identified.
[c] Less than 0.5 per cent.
[d] *Nigrospora, Helminthosporium, Epicoccum, Coniothyrium, Stemphylium, Curvularia, Rhizopus, Hormodendrum, Sphaeropsis,* and the blackpatch fungus (see literature citation 19) were isolated both years from both first- and second-year plants but none exceeded 0.5 per cent of the total isolates. *Chaetomella, Geotrichum, Thielavia, Trotteria, Monilia,* and *Zythia* were isolated only in 1951 from second-year plants. A few cultures of a fungus which appeared to be *Verticillium* were isolated in 1951. *Sclerotinia trifoliorum* was not found during this survey.

are not included in the summary tables for the state as they were not considered representative of the Madison area. Instead, data from other Madison fields were included. The kinds and relative prevalence of fungi isolated from all samples, exclusive of the Madison-A samples, in 1950 and 1951 are given in Table 3. The kinds and relative prevalence of the fungi isolated in the 2 years were remarkably similar even though the average severity of the rots was much greater in 1950 than in 1951. *F. oxysporum* was the most prevalent fungus, followed by *F. solani* and *F. roseum*. These 3 species comprised 66 per cent of the total isolates in 1950 and 68 per cent in 1951.

Early in these studies it became apparent that the relative prevalence of the fungi associated with diseased roots varied with age of plants, location, season of the year, cultural practices, and other factors. It was also observed that the microflora of the various parts of the root system of the same plant were not the same. Consequently, an attempt was made to evaluate the effects of these factors.

Effect of age of plant.—The relative prevalence of *Fusarium oxysporum* was higher on first-year plants than on second-year plants (Tables 3 and 4). Moreover, in first-year stands, it usually was highest on the youngest plants and tended to decrease as plants became older (Tables 2 and 5). This tendency was not so apparent in second-year stands (Table 5). *F. solani* was more prevalent on second-year plants than on first-year plants (Table 3). *Pythium* spp. were most commonly isolated from very young plants (Table 5).

Effect of location.—The relative prevalence of the fungi most commonly isolated from roots grown at the 6 locations sampled is given in Table 4. In general, the differences between these locations were not so great as might be expected. Perhaps the greatest differences occurred at Hancock where *Fusarium solani* was much less prevalent on both first- and second-year plants than at other locations. Also *F. oxysporum* and *F. roseum* were more frequently isolated from Hancock roots than from those from any other location. On first-year roots, *F. solani* was most prevalent at Ashland. *Gliocladium roseum* was most frequently isolated from plants grown at Madison and Sturgeon

TABLE 4.—*Relative prevalence of fungi most commonly isolated from diseased red clover roots of first- and second-year plants grown at 6 locations in Wisconsin in 1950 and 1951*[a]

Fungus	Age of Plants[b]	Percentage of total isolates from specified locations Ashland	Hancock	Madison	Marshfield	Spooner	Sturgeon Bay
Fusarium oxysporum	1	34	45	34	43	40	35
	2	22	40	29	30	35	27
Fusarium solani	1	28	5	17	12	17	16
	2	31	3	33	36	16	32
Fusarium roseum	1	8	14	8	10	8	14
	2	16	22	11	11	13	14
Phoma spp.	1	3	6	6	2	4	2
	2	4	7	5	3	6	6
Rhizoctonia spp.	1	2	1	2	2	4	4
	2	6	2	4	3	4	3
Gliocladium roseum	1	1	1	6	1	1	7
	2	2	T[c]	2	2	1	2
Pythium spp.	1	2	1	1	0	2	2
	2	1	1	T	T	0	T
Miscellaneous	1	22	27	26	30	24	20
	2	18	25	16	15	25	16

[a] Data are averages for both years.
[b] 1 = first-year plants; 2 = second-year plants.
[c] Less than 0.5 per cent.

TABLE 5.—*Relative prevalence of the fungi most commonly isolated from diseased roots of first- and second-year plants of red clover at various times during the growing season* [a]

Fungus	Percentage of total isolates								
	First-year stands				Second-year stands				
	June 20[b]	July 21	Sept. 13	Oct. 24	May 24[c]	June 25	July 27	Sept. 10	Oct. 25
Fusarium oxysporum	40	47	40	38	23	32	33	29	31
Fusarium solani	8	16	18	15	11	25	26	34	22
Fusarium roseum	16	10	8	10	24	19	12	11	14
Phoma spp.	4	4	8	10	5	4	5	4	4
Rhizoctonia spp.	2	1	3	2	6	4	3	3	2
Gliocladium roseum	1–	1	3	2	1	1	1	2	6
Pythium spp.	3	1	1	1	1	T[d]	T	T	T
Miscellaneous	26	20	19	22	29	15	20	17	21

[a] Data are averages for samples from 6 locations in Wisconsin for 1950 and 1951.
[b] Dates indicated are ± 7 days since all samples could not be plated on the same day.
[c] Data for May 24 are based on 1950 only as there were only 4 surveys in 1951.
[d] Less than 0.5 per cent.

Bay. This was particularly true in the case of first-year plants.

Effect of time of year.—In this survey, the effects of season of the year were confounded with the effects of age of plants, since the plants were not the same age at the various times of sampling. However, *Fusarium roseum* was isolated most frequently during the early part of the growing season; this was true for both first- and second-year plants (Table 5). *F. solani* was least prevalent early in the growing season and increased in prevalence during the summer and early fall months. *Gliocladium roseum* was most frequently isolated during the latter part of the growing season. *Pythium* spp. were most prevalent early in the season.

Effect of cultural practices.—Wide differences in the severity of root and crown rots between fields of red clover grown in the same locality on the same soil type were observed. It appeared probable that many of these differences were due to differences in cultural practices and management. Only preliminary work on this phase has been done in Wisconsin. Also, only preliminary investigations have been made on the effects of cultural practices on microflora. In 1949, 1950, and 1951, the relative prevalence of *Fusarium oxysporum*, *Gliocladium roseum*, *Rhizoctonia* spp., and *Pythium* spp. was higher on land cropped continuously to red clover than on land where a corn–small grain–legume rotation had been practiced.

Microflora of various parts of root system.—Tissues from the cortical and vascular areas of primary and secondary roots were plated separately and the data obtained are summarized in Table 6. *Fusarium oxysporum* was the most prevalent fungus in all tissues, was approximately equally abundant in the cortical areas of primary and secondary roots, was more prevalent in all tissues from first-year plants than from second-year plants, and was especially abundant in the vascular region of first-year plants. It was twice as prevalent in the latter as in the vascular region of second-year plants. *F. solani* and *F. roseum* were isolated from all parts of the root system, were most frequently isolated from second-year plants, and were least frequently obtained from the vascular tissues of first-year plants. Again, there was little difference between the relative prevalence of either of these species in primary and secondary roots of plants of the same age. Both species were common in the vascular tissues of second-year plants. *Phoma* spp. were isolated from all tissues. *Rhizoctonia* spp. were most frequently isolated from cortical tissue, especially cortical tissue of the primary roots. They were never isolated from the vascular core of first-year plants and only occasionally from this region in second-year plants. *Gliocladium roseum* was most commonly found in the

TABLE 6.—*Relative prevalence of the fungi isolated from cortical and vascular areas of primary and secondary roots of first- and second-year plants of red clover grown at 6 locations in Wisconsin in 1950 and 1951* [a]

Fungus	Percentage of total isolates from:					
	Cortical tissue				Vascular tissue	
	Primary roots		Secondary roots		Primary and secondary roots [b]	
	1[c]	2	1	2	1	2
Fusarium oxysporum	40	31	42	35	50	26
Fusarium solani	16	29	14	24	9	20
Fusarium roseum	10	14	12	14	5	22
Phoma spp.	8	5	8	6	8	7
Rhizoctonia spp.	2	6	2	3	0	1
Gliocladium roseum	3	2	1	1	4	4
Pythium spp.	1	1	1	1	0	0
Miscellaneous	20	12	20	16	24	20

[a] Data are averages for both years from all locations.
[b] Sections from vascular region of primary and secondary roots were bulked.
[c] 1 = first-year plants; 2 = second-year plants.

vascular tissues. *Pythium* spp. were isolated only from cortical tissues but were present in both primary and secondary roots.

DISCUSSION.—Root and crown rots constitute a major disease problem on red clover in Wisconsin. They are present in every field every year and can attack plants in any stage of development. They usually cause greatest damage when the plants are low in vigor or have been injured by adverse weather conditions or by insects. They are commonly severe in fields located on acid or infertile soils and on land continuously reseeded to clover without the intervention of other crops. While the most conspicuous losses involve the killing of plants, much damage occurs from reduced production by plants that are injured but not killed. There are all degrees of injury. The root systems become infected early and these infections spread and become progressively more severe. New infections are continuously being added to the older ones. Consequently, most plants sooner or later have small or large areas of their root systems damaged or destroyed and never attain their maximum potential productiveness. The relative importance of the fungi isolated may or may not be related to their relative prevalence on diseased plants. This can only be decided after adequate pathogenicity studies have been made. Such studies are in progress at Wisconsin and the results will be reported in later papers.

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DECAY OF BEECH WOOD BY THE HAPLOID AND DIPLOID MYCELIA OF ELFVINGIA APPLANATA [1]

Kiyowo Aoshima

SUMMARY

Wood-rotting ability of monosporous mycelia as well as diploid ones of *Elfvingia applanata* (pers.) Karst, was genetically analyzed.

Ability to cause decay of wood of monosporous mycelia of this fungus varied extensively according to their individual characteristics. Furthermore, no correlation existed between their known sexual factors and their ability to cause decay of wood. Numbers of monosporous mycelia as plotted against their ability to cause decay of wood followed a normal distribution curve, provided that they were derived from basidiospores of the same fruit body.

Eight diploid mycelia isolated from tissues of each of 8 fruit bodies of this fungus varied in ability to cause decay of wood. Variations in ability to cause decay of wood existing among these isolates were demonstrated to be the results of individual variation and not correlated with any host and locality influence.

There was also variation of ability to cause decay of wood among the diploid mycelia derived from pairing monosporous mycelia from the basidiospores of the same source. Numbers of diploid mycelia as plotted against their ability to cause decay gave a normal distribution curve. No significant difference of ability to cause decay of wood existed between the 2 types of diploid mycelia, providing all the haploid mycelia were derived from the same fruit body.

Diploid mycelia derived from 2 monosporous mycelia having pronounced ability to decay did not consistently cause pronounced decay. Results indicated that the differences in ability to cause decay existing among the diploid mycelia derived from the basidiospores of the same source, are the result of natural fluctuation.

Diploid mycelia from 2 paired monosporous mycelia obtained from a fruit body whose isolate had strong ability to cause decay also caused high decay loss. Diploid mycelia derived from 2 monosporous mycelia one from a sporophore whose isolate had strong ability to cause decay and the other from one of weak ability, are intermediate in their ability to cause decay, in relation to those of the diploid isolates indicated above.

The reason for the difference in ability to cause decay between haploid and diploid mycelia is not clear in this species possibly because of difficulties in obtaining a satisfactory method of comparing decay ability. However the variation range of haploid mycelia, within the same source, is obviously greater than that of the diploid mycelia.

There have been many investigations of wood-decay by the fungus *Elfvingia applanata* (Pers.) Karst. (*Fomes applanatus*), in the field as well as in the laboratory, in many countries. The fungus which has a wide distribution, damages many species of broad-leaved trees and, less frequently, of coniferous trees both standing and felled.

Most investigations on wood-decay caused by various species of wood-rotting fungi, including this fungus, have been carried out by the pure culture method, using diploid mycelia isolated from tissue of the fruit body, from rot, or from basidiospores.

The writer (1) has previously indicated that the basidiospores of *Elfvingia applanata* have a long dormant period and that high temperature treatment induced earlier germination. It has also been established (2) that the fungus is a heterothallic and a tetrapolar species. In the present paper, wood-rotting abilities of haploid as well as diploid mycelia of *Elfvingia applanata* will be genetically analized.

DECAY BY HAPLOID MYCELIA. — *Materials and methods.*—Single spore isolates used in the present experiment were identical with those used previously (1, 2), being obtained from 2 fruit bodies growing on different hosts. The sexual factors of the 2 fruit bodies were designated $A_1A_2B_1B_2$ and $A_1A_3B_2B_4$, respectively. Decay tests were made in glass bottles (18 cm high, 8 cm in diameter), each containing 40 gm of sawdust of beech wood, 20 gm of rice bran and 100 cc of water. Four test blocks of the outer sap-wood of *Fagus crenata*, 6 × 1.5 × 1 cm were placed beneath this medium in a bottle. These test blocks had first been oven dried and weighed. After sterilization for 30 minutes at 15 lb. steam pressure, the test bottles were seeded with mycelium from each agar culture of monosporous mycelia of *Elfvingia applanata* and kept in a culture room at temperatures from 20 to 28°C. for 6 months (April 5-October 5, 1952). Three weeks after inoculation, most of the haploid mycelia developed in the media and grew over the wood blocks. On October 6, the wood blocks were removed from the bottles, oven dried and weighed. Percentage of loss in weight was then calculated. Some of the haploid mycelia grew in the media but did not cover the wood blocks. As these wood blocks appeared not to have been invaded equally on the upper and underside by the mycelia, they were excluded from the calculations.

Results.—Fig. 1 shows the percentage of loss in weight[2] of these wood blocks rotted by the haploid

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[2] An average of 4 test blocks, equal in the latter experiments.

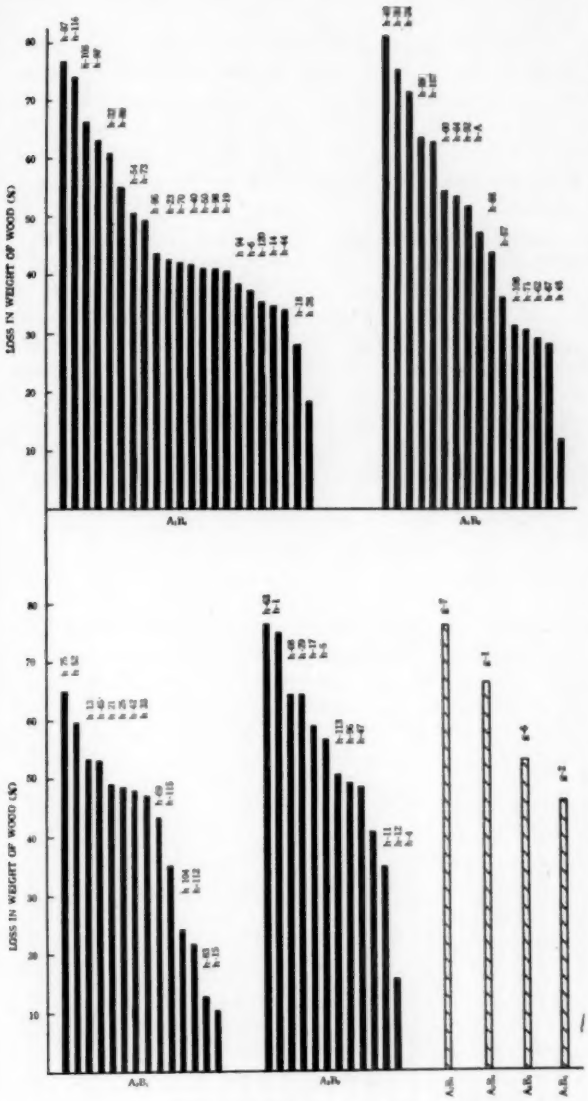

FIG. 1. Percentage of loss in weight of wood-blocks decayed by the haploid isolates derived from the basidiospores of fruit body (h) and fruit body (g) of *Elfvingia applanata* (pers.) Karst.

mycelia derived from 2 fruit bodies[3] arranged by sexual factors. The ability to cause decay of wood of each of the 64 haploid mycelia derived from the same fruit body varied widely. Monosporous isolate, No. h-49 destroyed the 4 wood blocks most vigorously and their average percentage of loss in weight reached 82.20, whereas monosporous isolate, No. h-15 gave an average of only 10.39 per cent loss. It is true in haploid mycelia of g-group. This figure also shows the lack of any relationship between sexual factors of haploid mycelia and their rotting ability.

Fig. 2 shows the number of haploid mycelia plotted against their ability to cause decay, as indicated in percentage of loss in weight of wood blocks. From this curve it can be seen that variation of rotting ability of wood between each of the monosporous mycelia from the same fruit body is merely a fluctuation.

DECAY BY DIPLOID MYCELIA.—*Materials and methods.*—Forty-seven diploid mycelia were obtained by pairing 2 mating types of haploid mycelia used in the preceding experiments. Also, 8 diploid mycelia isolated by the writer from various localities in Japan were employed as follows: Isolate (a), tissue culture from fruit body on *Fagus crenata,* Minamitsuru-gun, Yamanashi pref., Oct., 1949; Isolate (c), tissue culture from fruit body on *Betula ermanii communis,* Kamikochi, Nagano pref., Aug., 1951; Isolate (d), tissue culture from fruit body on *Betula ermanii communis,* Kamikochi, Nagano pref., Aug., 1951; Isolate (e), tissue culture from fruit body on *Betula ermanii communis* Kamikochi, Nagano pref., Aug., 1951; Isolate (g), tissue culture from fruit body on *Quercus myrsinaefolia,* Meguro, Tokyo, June, 1951; Isolate (h), tissue culture from fruit body on *Quercus myrsinaefolia,* Meguro, Tokyo, June, 1951; Isolate (j), tissue culture from fruit body on *Fagus crenata,* Nakatsuchi, Kitaazumi-gun, Nagano pref., Aug., 1951; Isolate (k), tissue culture from fruit body on *Quercus sp.,* Koishikawa, Tokyo, Sept., 1948.

The method of determining ability of the diploid mycelia to cause decay and the date of beginning and end of this experiment were the same as in the preceding experiment.

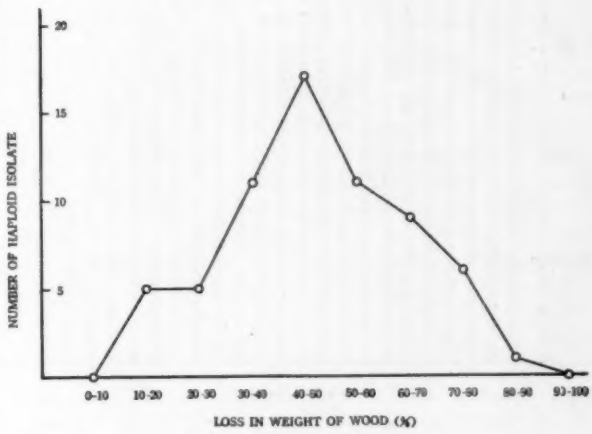

FIG. 2. Relation of percentage of loss in weight of wood-blocks and number of haploid isolates from fruit body (h).

[3] Tissue culture from these 2 fruit bodies are designated as Isolate (h) and Isolate (g), respectively.

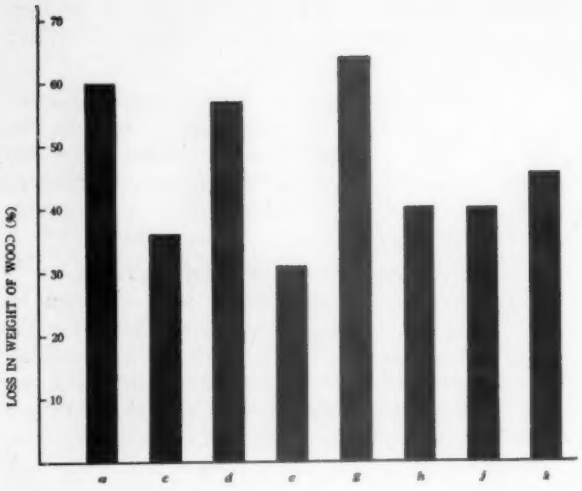

FIG. 3. Percentage of loss in weight of wood-blocks of 8 diploid mycelia isolated from tissues of each of 8 fruit bodies on different hosts and from various localities in Japan.

Results.—Fig. 3 shows the percentage of loss in weight of wood blocks attacked by these 8 cultures of diploid mycelia isolated from tissues of each fruit body.

From this figure it may be noted that, among the 8 isolates, there exist some significant differences in ability to cause decay. It is also shown that 3 cultures (c, d, e) isolated from sporophores on the same host species in the same year at the same locality but not from the same tree, vary in their ability to cause decay of wood.

Forty-four diploid mycelia derived from the pairing of monosporous mycelia used in the preceding experiment from fruit body (h), 2 from pairing haploid mycelia of fruit body (g) and 1 from pairing haploid mycelia of fruit body (h) and fruit body (g) were tested for ability to cause decay of wood. Fig. 4 shows the percentage of loss in weight of wood blocks of *Fagus crenata* caused by these mycelia arranged separately by sexual factors ($A_1\ B_1 \times A_2\ B_2$, $A_1\ B_2 \times A_2\ B_1$, $A_3\ B_4 \times A_1\ B_3$, $A_1\ B_4 \times A_3\ B_3$, $A_3\ B_4 \times A_1\ B_1$). In this case there was also considerable variation in

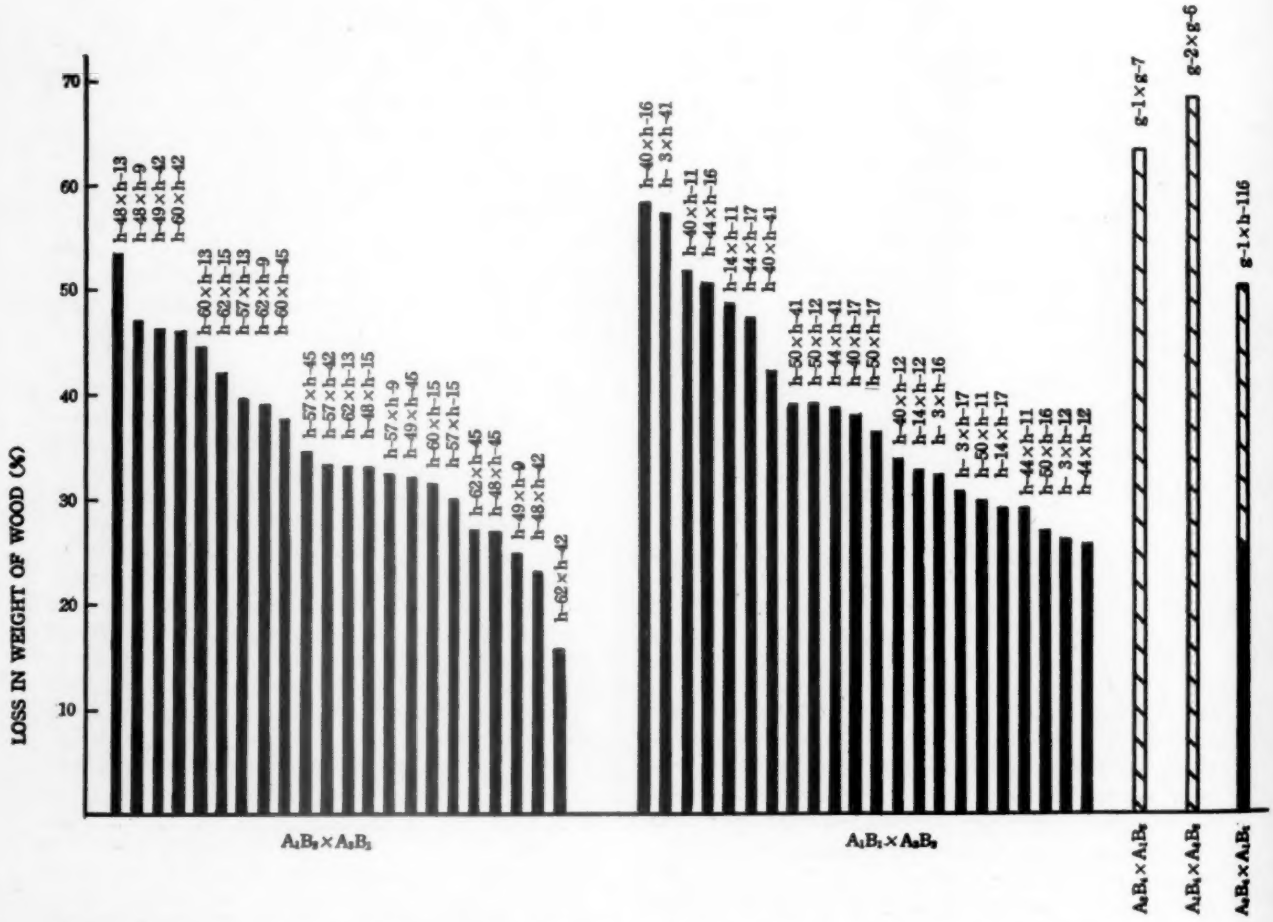

FIG. 4. Percentage of loss in weight of wood-blocks destroyed by the diploid mycelia derived from pairing of monosporous mycelia of fruit body (h) and fruit body (g).

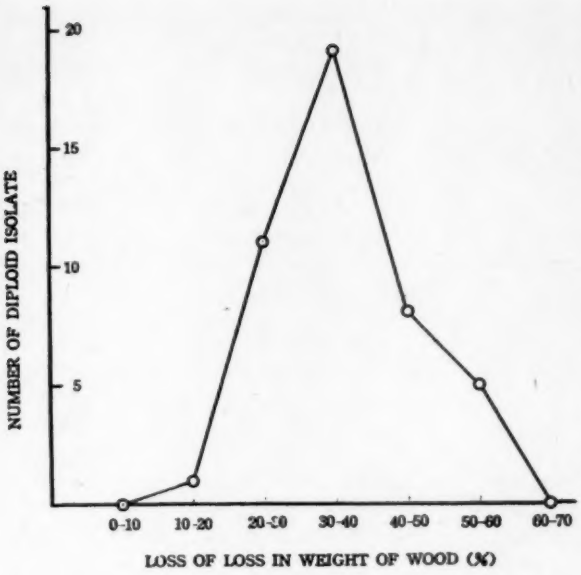

FIG. 5. Relation of percentage of loss in weight of wood-blocks and number of diploid isolates from fruit body (h).

ability to cause decay of wood. From this figure, it will be seen that there is no significant correlation between the sexual factors of haploid mycelia from which the diploid mycelia have been derived and the ability of the resulting diploid mycelia to cause decay of wood if the diploid mycelia have the same composition of sexual factors ($A_1 A_2 B_1 B_2$).

Diploid isolate No. h-40 × No. h-16 was foremost in woodrotting ability, with 57.63 per cent, while No. h-62 × No. h-42 was the least with 15.90 per cent. Fig. 5 shows the relation between the number of diploid isolates and their ability to cause decay of wood. From this it can be concluded that the variation of ability to cause decay of wood existing among the diploid mycelia from the same fruit body is a fluctuation. Furthermore, the diploid isolate, No. h-49 × No. h-45 is not so capable in causing wood decay (32.49 per cent) as were haploid isolates, No. h-49, the strongest of all the haploid mycelia with 82.20 per cent, and No. h-45, a moderately strong decayer with 53.51 per cent.

No. h-49 × No. h-42 (46.45 per cent) is stronger in ability to cause decay than No. h-49 × No. h-45 (32.49 per cent), where No. h-42 (48.44 per cent) is weaker in ability to cause decay of wood than No. h-45 (53.51 per cent).

No. h-15 × No. h-62 is strong with 42.25 per cent, where No. h-15 is the weakest of all the haploid mycelia with 10.39 per cent and No. h-62 is moderately weak with 29.29 per cent.

No. h-15 × No. h-60 (31.46 per cent) is weaker in ability to cause decay than No. h-15 × No. h-62 (42.25 per cent), where No. h-60 (54.80 per cent) is much stronger than No. h-62 (29.29 per cent).

Ability to cause decay of wood of the 2 haploid mycelia, No. g-1 and No. g-7 is greater than those of No. g-2 and No. g-6, respectively. On the other hand, wood-rotting ability of the diploid mycelium, No. g-1 × No. g-7 (62.80 per cent) is less than that of the diploid mycelium, No. g-2 × No. g-6 (67.99 per cent). These results suggest that ability of diploid mycelia to cause decay within the same source, is not indicated by the decay ability of the parent monosporous mycelia.

Both diploid mycelia from fruit body (g) caused greater weight loss of wood blocks than any of the diploid mycelia derived from the fruit body (h). The percentage of loss in weight for the 2 diploid mycelia, No. g-1 × No. g-7 and No. g-2 × No. g-6 amounted to 62.80 and 67.99 per cent, respectively.

Two diploid mycelia isolated from the tissues of each of the 2 fruit bodies, Isolate (g) and Isolate (h)[4] differ in their ability to cause decay (Fig. 3). Isolate (g) caused 64.23 per cent loss in weight and Isolate (h) 40.19 per cent. This difference is significant at the 5 per cent level. Comparing this with results presented above, it can be concluded that the decay ability of diploid isolates derived from tissue of fruit body, is indicative of the ability of the diploid mycelia derived from the basidiospores to cause decay.

The diploid mycelium derived from the 2 basidiospores of different fruit bodies, No. g-1 × No. h-116 caused 49.68 per cent in loss in weight of wood blocks. This value is moderately strong compared with that of the diploid mycelium derived from the fruit body (h) (40.19 per cent), but weak compared with that from the fruit body (g) (64.23 per cent). Haploid mycelium, No. g-1 has a wood-rotting ability of 66.42 per cent and No. h-116 that of 74.33 per cent. These 2 monosporous mycelia both have pronounced ability to cause decay, but the diploid mycelium derived from the pairing of these 2 monosporous mycelia (one from the fruit body whose isolate has strong ability and another from that of weak ability) caused only moderate decay of wood.

Growth of haploid isolate No. h-22 and decay of wood blocks by haploid and diploid isolates is illustrated in Fig. 6.

DISCUSSION.—Observation on wood decay by the haploid mycelia of wood-rotting Basidiomycetes was reported for the first time by Verrall (11). He has reported that among the haploid cultures of *Phellinus* (*Fomes*) *igniarius* (L.) Quél. from birch, the wood decaying proclivities did not vary significantly, although there were differences in rate of growth on malt agar. The haploid cultures from aspen and ironwood, however, vary considerably in the amount of decay caused. Mounce and Macrae (8) observed that some of the monosporous mycelia of *Fomitopsis* (*Fomes*) *pinicola* (Schw.) Karst. destroyed the wood blocks of *Picea sitchensis* fairly rapidly, but others caused very little decay. These results are rather similar to that of the writer's experiment in which it is indicated that a large variation exists between the wood-rotting ability

[4] Isolate (g) is designated as $A_1 A_2 B_3 B_4$ and isolate (h), $A_1 A_2 B_1 B_2$.

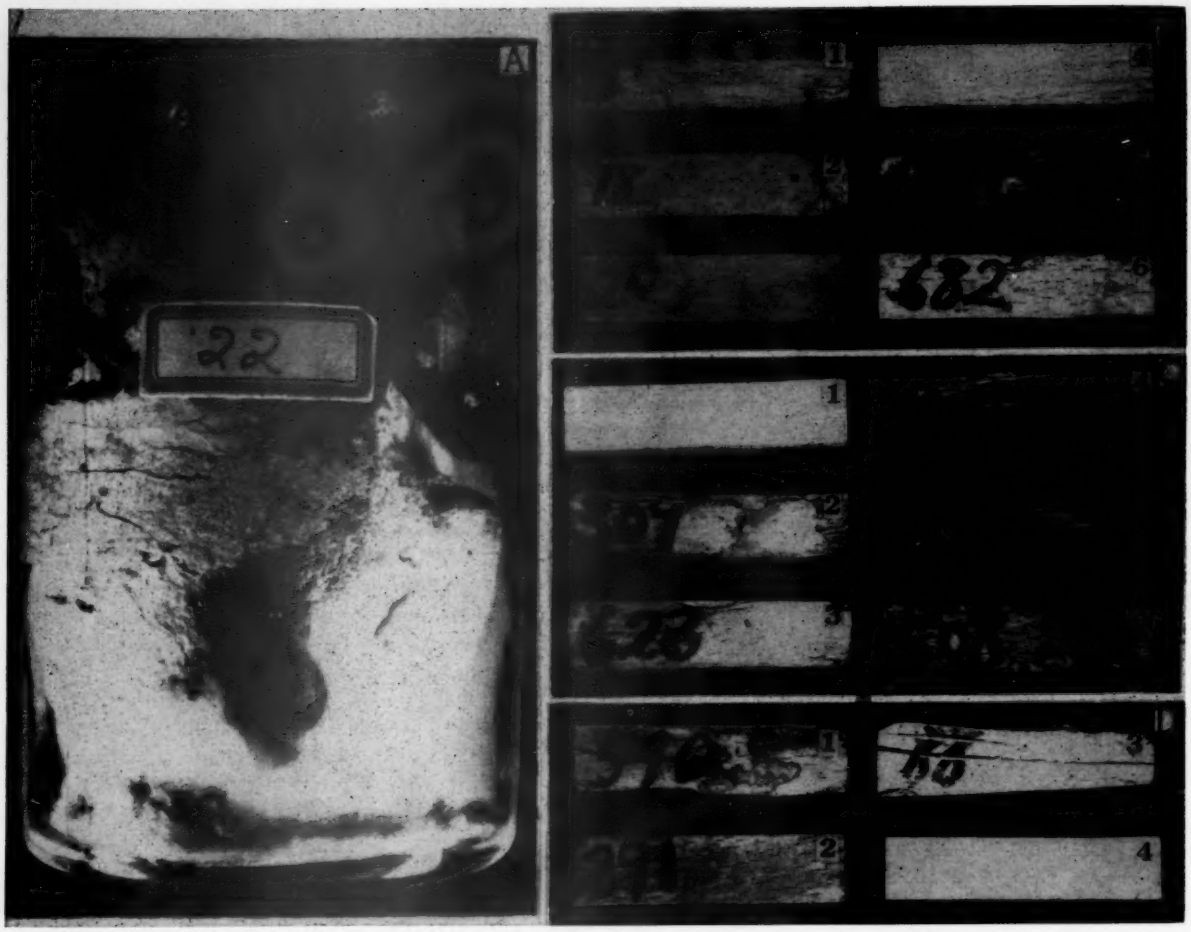

FIG. 6. A) Mycelia of haploid isolate No. h-22 in a bottle at the end of decay experiment. B-D) Wood-blocks of *Fagus crenata* decayed by the haploid and diploid isolates; B, 1) Haploid isolate, No. h-15; 2) Haploid isolate, No. h-62; 3) Diploid isolate, No. h-15 × No. h-62; 4) Control; 5) Diploid isolate, No. h-50 × No. h-16; 6) Diploid isolate, No. h-60 × No. h-15. C; 1) Control; 2) Haploid isolate, No. h-45; 3) Diploid isolate, No. h-45 × No. h-49; 4) Haploid isolate, No. h-92; 5) Haploid isolate No. h-92; 6) Diploid isolate, No. h-42 × No. h-49. D; 1) Haploid isolate, No. g-6; 2) Haploid isolate No. g-2; 3) Diploid isolate, No. g-2 × No. g-6; 4) Control.

of haploid mycelia of *Elfvingia applanata*.

The writer has indicated that the haploid mycelia of *Elfvingia applanata* destroyed the wood blocks of *Fagus crenata* considerably, but that the wood-rotting ability of each of 64 haploid mycelia differed greatly ranging from 10.39 to 82.20 in percentage of loss in weight of wood blocks. From Fig. 1 it is evident that no correlations exist between wood-rotting ability of haploid mycelia and their sexual factors.

As shown in Fig. 2, the numbers of haploid isolates as plotted against wood-rotting ability follow the normal distribution curve.

It has been well established by several investigators that there is variation of wood-rotting ability among the diploid isolates. Schmitz (10) found differences of wood-rotting ability among 4 isolates of *Fomitopsis* (*Fomes*) *pinicola*, and concluded that in this species physiological variation existed which might be the results of host influence.

Mounce (7) worked with the same fungus and concluded that the difference was the result of individual variation rather than host influence. Owens (9) also found that there existed variation of ability to cause decay among the isolates of *Cryptoderma* (*Fomes*) *pini* (Thore) Imaz. Verrall (11) investigated *Phellinus* (*Fomes*) *igniarius* and found that there existed variation of wood-rotting ability among the isolates. He also stated that there were 3 distinct groups in this species, which differ among themselves in morphological as well as physiological characteristics and in ability to cause decay of wood from the standpoint of host relationship. Childs (3) found that different isolates of *Phaeolus* (*Polyporus*) *schweinitzii* (Fr.) Pat. varied from one another in ability to cause decay of wood. Hilborn (4) pointed out that the isolates of *Fomes fomentarius* (L.) Kickx differed among themselves in their ability to cause decay of wood and that this was the result of individual variation and not corre-

lated with any host or locality influence.

Differences in wood-rotting ability exist among 8 isolates of *Elfvingia applanata* (Fig. 3). Three of these were isolated from tissues of each of the fruit bodies grown on the same host (*Betula ermanii communis*) at the same locality but not on the same tree, and collected on the same date. There is also difference in ability to cause decay among these 3 isolates. Two of 8 isolates from the same host and locality were also different in their ability to cause decay. It can therefore be concluded that the difference in ability to cause decay of wood existing among the isolates of *Elfvingia applanata* is the result of individual variation, and not correlated with any locality influence. There is also considerable variation in ability to cause decay of wood among 44 diploid mycelia derived from the basidiospores from the same source. They range from 56.63 to 15.90 per cent in loss in weight of wood blocks. This range of variation is smaller than that of haploid mycelia.

Furthermore, the writer has pointed out that there exists no significant difference in ability to cause decay of wood between the 2 types of diploid mycelia ($A_1 B_1 \times A_2 B_2$ and $A_1 B_2 \times A_2 B_1$).

Diploid mycelia derived from 2 monosporous mycelia, both with strong decaying ability, do not always have much ability to cause decay of wood. Similarly, it was also ascertained that diploid mycelia from the monosporous mycelia, both with weak decaying ability do not always have weak ability to cause decay of wood. The considerable difference of ability to cause decay existing among the diploid mycelia can not be explained on the basis of the decay ability of the haploid strains from which they were derived.

Two diploid mycelia from the basidiospores of another fruit body, (g), (Fig. 4) have more ability to cause decay than any of the diploid mycelia from the basidiospores of fruit body (h), while the 4 monosporous mycelia from fruit body (g) have no greater ability to cause decay than many of the monosporous mycelia from fruit body (h).

Diploid mycelia derived from 2 basidiospores, one from fruit body (h) and the other from fruit body (g), have wood-rotting ability intermediate between that of diploid mycelia from fruit body (h) and from fruit body (g). Here, it must be mentioned that wood-rotting ability of Isolate (g) is significantly greater than that of Isolate (h). From the fact mentioned above, it can be concluded that the wood-rotting ability of diploid mycelia is inherited from the parent diploid mycelia, regardless of the ability to cause decay of 2 mating types of monosporous mycelia.

Verrall (11) has reported in his study on variation in *Phellinus igniarius* that the haploid cultures of this fungus decay wood more slowly than the dicaryotic tissue cultures of the parent fruiting bodies. Kaufert (6) has reported that the dicaryotic mycelia of *Pleurotus corticatus* Fr. decay basswood sawdust more rapidly than haploid mycelia in tests lasting 90-150 days. When the decay period was lengthened to 210 days, however, the haploid mycelia caused as much decay as the parent dicaryotic mycelium or compatible matings of haploid mycelia. Ito (5) has reported that haploid mycelia of *Fomitopsis* (*Fomes*) *rhodophaea* (Lév.) Imaz. destroyed the wood blocks of *Liriodendron tulipifera* as quickly as the diploid mycelia.

Considerable difference existed (Fig. 2, 5) between the wood-rotting ability of haploid and diploid mycelia of *Elfvingia applanata*, if they are derived from the same source. The diploid mycelia grew on the wood blocks more rapidly and compactly than the haploid mycelia, covering the wood blocks with brownish black and felty mycelia. Therefore, it is possible that the oxygen supply to the wood blocks is somewhat inhibited in the case of diploid mycelia. For this reason comparison cannot be made between the ability of haploid mycelia to cause decay and that of diploid mycelia without some chance of error. However, it can be pointed out that the variation range of ability to cause decay of haploid mycelia (10.39-82.20 per cent) is much greater than that of the diploid mycelia (15.90-57.63 per cent).

GOVERNMENT FOREST EXPERIMENT STATION
MEGURO, TOKYO, JAPAN

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INHERITANCE OF RESISTANCE TO STEM RUST AND POWDERY MILDEW IN CYTOLOGICALLY STABLE SPRING WHEATS DERIVED FROM TRITICUM TIMOPHEEVI [1]

R. W. Allard and R. G. Shands[2]

SUMMARY

Two hard red spring wheat selections, C.I. 12632 and C. I. 12633, were derived from an interspecific hybrid between a common hard red spring wheat and *Triticum timopheevi* by 2 generations of backcrossing to the common wheat parent followed by 8 generations of selfing. These 2 cytologically stable selections possess a high order of adult plant resistance to stem and leaf rusts and mildew, and a necrotic type of resistance to mildew in the seedling stage. C. I. 12633, in addition, is highly resistant to loose smut.

The stem rust resistance of these selections in hybrids with Reward and Marquis is governed by dominant duplicate genes which are linked with a recombination value of 14.78 ± 1.75 per cent. Adult plant resistance to stem rust and adult plant resistance to powdery mildew were so closely associated that no unquestioned recombinations occurred in 762 F_3 progenies. This result would be expected if 2 genes govern adult mildew response, 1 very closely linked with each of the 2 genes conditioning resistance to stem rust. However, the possibility that the resistances may result from pleiotropic effects of the same 2 Mendelian units could not be excluded, nor the possibility that several genes for mildew resistance may be situated in the chromosome segment in question. In addition to the genes governing adult plant response to mildew, C. I. 12632 and C. I. 12633 carry a dominant allele for necrotic response to mildew which influences the mildew infection of the first seedling leaf appreciably and to a lesser extent that of the higher leaves, as well as 1 or more other genes that influence mildew reaction.

C. I. 12632 and C.I. 12633 appear to be promising sources of resistance to stem and leaf rusts and to mildew. C. I. 12633 may also be of value as a source of resistance to loose smut.

Timopheevi wheat (*Triticum timopheevi* Zhuk.) with 14 haploid chromosomes has been used successfully by at least 3 plant breeders (1, 7, 11) in crosses designed to transfer its stem and leaf rust resistance to common wheat types even though its hybrids with *T. vulgare* Vill. (*T. aestivum* L.) are highly sterile. The variety Timstein, developed by Pridham, has been shown by Semeniuk (10) to vary in meiotic stability, but it includes cytologically stable lines. Sears and Rodenhiser (9) found the resistance of Timstein to races 17 and 56 of stem rust, caused by *Puccinia graminis tritici* Eriks. & E. Henn., to be governed by linked complementary genes located on chromosome X. Koo and Ausemus (3) also investigated the inheritance of the stem rust resistance of Timstein.

Shands (11) transferred the stem rust resistance of *T. timopheevi* to hard red winter wheat selections but these are limited in usefulness by an association between resistance and late maturity. Resistance to leaf rust caused by *Puccinia rubigo-vera tritici* (Eriks.) Carleton was transferred in the same cross.

Timopheevi disease resistance also was transferred by Allard (1) who described stem and leaf rust resistant lines that had 42 chromosomes and were cytologically stable. Additional selections have since been made in these stocks. The origin, agronomic characteristics and cytological behavior, and the inheritance and interrelation of resistance to stem rust and powdery mildew of 2 of these selections, C. I.[3] 12632 and C. I. 12633, are reported here.

ORIGIN OF C. I. 12632 AND C. I. 12633.—The common wheat parent of the interspecific hybrid was an unnamed hard red spring wheat strain designated 2666A2-2-15-6-3 by the Wisconsin Agricultural Experiment Station, which was derived from the cross, Illinois No. 1 × Chinese C. I. 6223. The other parent was *T. timopheevi*, P. I.[4] 94761. The hybrid was backcrossed twice by pollination with the common wheat parent. Several cytologically stable 42-chromosome plants, which were also early in maturity, rust resistant, and acceptable agronomically, appeared in the F_2 generation of the second backcross. Several of the F_3 progenies derived from these plants were homozygous for rust resistance. Selection for desirable agronomic type was continued in these lines until the seventh selfed generation, when the lines appeared to be homozygous. The final single plant selections, later designated C. I. 12632 and C. I. 12633, were made in the eighth selfed generation. These selections were derived from different second backcross F_2 plants.

AGRONOMIC CHARACTERISTICS.—At Madison, Wisconsin, C. I. 12632 and C. I. 12633 head about 1 day later than Hope and Henry and about 3 days later than the Thatcher variety. At Davis, California, their heading

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[3] C. I. refers to the accession number of the Division of Cereal Crops and Diseases, B. P. I. S. A. E., A. R. A., U. S. D. A.

[4] P. I. refers to the accession number of the Division of Plant Exploration and Introduction, B. P. I. S. A. E., A. R. A., U. S. D. A.

date is about the same as that of Baart. Both selections are considered midseason in heading date. Four year average yields in replicated small plot tests at Madison were 25.5, 23.1, 33.1, and 20.3 bushels per acre for C. I. 12632, C. I. 12633, Henry, and Thatcher, respectively. At Davis in 1948, C. I. 12632, C. I. 12633, Hope, and Baart yielded 60.2, 57.0, 42.4, and 74.4 bushels per acre, respectively. Thus, their yields have been superior to Hope but somewhat less than those of best yielding commercially grown varieties. The straw of the 2 selections is moderately weak and the kernels are small. Both selections have weak glumes, leading to severe shattering under the low humidities which prevail at Davis. For grain production, C. I. 12632 probably is slightly superior to C. I. 12633. Populations from hybrids of the 2 selections crossed with other varieties have been free or nearly free from the brown necrosis characteristic of varieties deriving their stem rust resistance from Hope.

DISEASE REACTION.—Although attention was limited to stem and leaf rust resistance during the breeding of these selections, the resistance of *T. timopheevi* to 2 other major diseases of wheat was also recovered. C. I. 12632 possesses a high order of resistance to powdery mildew, caused by *Erysiphe graminis tritici* El. Marchal, in addition to resistance to stem and leaf rusts. It has shown moderate resistance to local collections of loose smut at Madison. C. I. 12633, in addition to being stem rust, leaf rust, and mildew resistant, is immune to all known races of loose smut except race 2, to which it is highly resistant.[5] However, little of the bunt resistance of Timopheevi wheat was transferred to these selections since both are only slightly less susceptible to race T-1 of bunt than their common wheat parent (8).

The races of *Puccinia graminis tritici* that occurred at Madison or Davis during the 8 years these selections have been under test are numbers 15B, 17, 19, 38, and 56.[6] During the same period races 2, 5, 9, 11, 15, 30, 37, 58, 65, 76, 80, 81, 104, 105, and 126 of *P. rubigovera tritici* appeared in 1 or more years.[6] Since no more than a trace of rust occurred on either selection during that period, they may be presumed to possess adult plant resistance to the above races of stem and leaf rusts.

When C. I. 12633 was inoculated with spores of a collection of race 15B *P. graminis tritici* in the greenhouse at Madison, a few large pustules developed in both seedling and adult stages. Since C. I. 12633 has been resistant in the field at some locations in both North and South America where race 15B was present and susceptible at others, its place in breeding for resistance to the 15B complex cannot be assessed yet. Little information is available concerning the reaction of C. I. 12632.

C. I. 12632 is susceptible in seedling stage to a number of races and C. I. 12633 to a few races of leaf rust.[7] Timopheevi wheat is resistant in seedling stage to all known races of leaf rust. It is apparent that not all of the seedling stage leaf rust resistance of that species was transferred to C. I. 12632 or C. I. 12633.

MEIOTIC BEHAVIOR.—Because of the possibility of cytological irregularities resulting from their interspecific origin, it was desirable to determine the cytological behavior of C. I. 12632 and C. I. 12633 before undertaking inheritance studies with them. Accordingly, they were hybridized with 4 varieties of common wheat, and meiosis was studied from acetocarmine smear preparations of pollen mother cells of the F_1 hybrids. Hybrids of Hope with 3 of the same varieties and the 7 parents involved also were studied. The numbers of univalents, bivalents, and multiple associations and the frequency of micronuclei in the quartets of microspores were recorded. Twenty-five pollen mother cells and 200 quartets of microspores were examined in each of 2 plants of each parent or F_1 hybrid. Since differences between the 2 plants were in no instance statistically significant, only the mean values for each parent or hybrid are presented (Table 1). Multiple associations were observed only sporadically and are not reported.

The differences among the 7 parental common wheat varieties were in no case significant. Hence, the means of the 7 parents were used as standards for comparisons in meiotic behavior between hybrids and nonhybrids (Table 1). In comparisons of that sort the F_1 hybrids were frequently more irregular cytologically than the parental varieties, whether the comparison was based on chromosome pairing or on the prevalence of micronuclei. Because 2666A2-2-15-6-3 occurred 3 times in the parentage of C. I. 12632 and C. I. 12633, it is not surprising that hybrids of these 2 strains with that parent were among the most regular cytologically of the F_1 hybrids. Hope, C. I. 12632, and C. I. 12633 are varieties of recent interspecific origin. However, their hybrids did not appear to be less regular cytologically than hybrids having as 1 parent Reward, Poso, White Federation, or 2666A2-2-15-6-3, which are not recently derived from interspecific hybridization. F_1 hybrids involving Hope and C. I. 12633 were possibly more irregular in cytological behavior than hybrids involving C. I. 12632.

It may be concluded that C. I. 12632 and C. I. 12633 are themselves cytologically regular and are as regular cytologically in their hybrids as a number of common wheat varieties, including Hope, that have not caused cytological difficulties when used as parents. Hence, inheritance studies with these 2 strains should not be upset by cytological disturbances.

[5] Data kindly furnished by W. M. Bever, Division of Cereal Crops and Diseases, B. P. I. S. A. E., U. S. D. A., Urbana, Ill.

[6] Isolated from uredial material obtained from the U.S.D.A. Cooperative Uniform Rust Nursery. Data kindly supplied for stem rust by E. C. Stakman and W. Q. Loegering, Rust Laboratory, St. Paul, Minn. Leaf rust data supplied by R. M. Caldwell, Purdue University Agricultural Experiment Station, Lafayette, Ind., and C. O. Johnston, U. S. D. A., Manhattan, Kans.

[7] Data kindly furnished by C. O. Johnston, U. S. D. A., Manhattan, Kans.

TABLE 1.—*The mean frequency per pollen mother cell of univalents and bivalents at meiotic metaphase I and the occurrence of micronuclei in the quartet stage in several common wheat varieties and F_1 hybrids grown at Davis, Calif. in the spring of 1948*

Parents or hybrids	Frequency per P.M.C. of: Univalents	Bivalents	Quartets with no micronuclei
	No.	No.	Per cent
Poso C. I. 8891	0.16	20.92	96.2
Hope C. I. 8178	0.16	20.92	93.5
Reward C. I. 8182	0.28	20.82	—
White Federation C. I. 4981.	0.20	20.90	93.8
C. I. 12632	0.20	20.90	92.8
C. I. 12633	0.28	20.84	94.2
2666A2-2-15-6-3	0.44	20.70	94.0
Mean of parents	0.25	20.86	94.1
2666A2-2-15-6-3 × C. I. 12632	0.32	20.84	92.0
2666A2-2-15-6-3 × C. I. 12633	0.52	20.74	84.8
Mean of hybrids with 2666A2-2-15-6-3	0.42	20.79	88.4
Reward × C. I. 12632	0.44	20.70	90.5
Poso × C. I. 12632	0.26	20.38	78.2*
White Federation × C. I. 12632	1.26**	20.30	84.0
Mean of hybrids with C. I. 12632	0.64	20.46	84.2
Reward × C. I. 12633	1.16**	20.46	93.2
Poso × C. I. 12633	0.52	20.04**	87.5
White Federation × C. I. 12633	1.42**	20.26*	81.5
Mean of hybrids with C. I. 12633	1.03*	20.25*	87.4
Reward × Hope	0.54	20.62	83.2
Poso × Hope	1.04*	19.54**	74.5**
White Federation × Hope	1.02*	20.48	75.5**
Mean of hybrids with Hope	0.87*	20.21*	77.7*
Reward × 2666A2-2-15-6-3	1.20**	20.38	74.2**
Poso × 2666A2-2-15-6-3	0.82	19.94**	79.8
White Federation × 2666A2-2-15-6-3	1.12**	20.44	78.0*
Mean of crosses between common wheat varieties	1.05*	20.25*	77.3*

* Significantly more irregular cytologically at odds 19:1 than the mean of 7 common wheat parents.
** At odds 99:1.

INHERITANCE OF STEM RUST RESISTANCE.—Studies of the inheritance of resistance were confined to the reaction of mature plants in the field. Infection was initiated by the inoculation of susceptible spreader rows early in the season. At Madison, races 17, 19, and 56 were used. At Davis, race 56 alone was present in 1946 and only race 17 in subsequent seasons. Race 15B was not present during the inheritance studies.

C. I. 12632 and C. I. 12633 in crosses with the susceptible varieties Reward and Marquis produced F_1 plants equal to their resistant parent in reaction to stem rust. In the F_2 and F_3 generations only individuals with the parental types of reaction occurred. The ratios of resistant to susceptible individuals observed in F_2 populations grown in several different seasons at both Davis and Madison are in Table 2. In each of the 8 F_2 populations the ratio of resistant to susceptible individuals exceeded 3:1. In 3 of the F_2 populations the deviation from a ratio of 3 resistant: 1 susceptible was too great to be explained by chance alone,

TABLE 2.—*The ratios of resistant to susceptible plants in F_2 populations of the hybrids of Reward and Marquis with C. I. 12632 and C. I. 12633, and goodness of fit to two factorial hypotheses*

Hybrid	Location and year	Stem rust reaction: Resistant	Stem rust reaction: Susceptible	Ratio Resistant Susceptible	Probability: 3:1 ratio	Probability: Duplicate linked factors with 14.78 per cent recombination
		No.	No.			
Reward × C. I. 12632	Madison 1947	272	43	6.33:1	<0.01	0.04
Reward × C. I. 12632	Madison 1948	127	33	3.85:1	0.20	0.43
Reward × C. I. 12632	Madison 1950	49	9	5.44:1	0.10	0.62
Reward × C. I. 12633	Madison 1947	286	62	4.61:1	<0.01	0.98
Reward × C. I. 12633	Madison 1948	189	47	4.02:1	0.08	0.67
Reward × C. I. 12633	Madison 1950	170	49	3.47:1	0.39	0.11
Reward × C. I. 12633	Davis 1947	247	67	3.69:1	0.14	0.16
Marquis × C. I. 12633	Davis 1947	276	67	4.12:1	0.02	0.49
Total		1616	377	4.29:1	<0.01	0.40

and the total for the 8 F_2 populations deviated significantly from a 3:1 ratio. When the F_2 data were classified by locations, years, and hybrids in a hierarchical table according to the method of Snedecor and Brandt, described by Fisher (2), the χ^2 for heterogeneity was nonsignificant. Thus, the 8 F_2 populations agree in indicating an excess of resistant individuals for a good fit to the 3:1 ratio expected under the hypothesis that a single dominant gene governs stem rust resistance.

The mean number of plants in the F_3 progenies reported in Table 3 was 52. The F_3 progenies do not conform to the ratio of 1 homozygous resistant:2 segregating:1 homozygous susceptible progeny expected with a single dominant gene. In addition, the majority of the F_3 lines which were segregating for rust resistance approximated the F_2 segregation, i.e., the ratio of resistant to susceptible plants was about 4:1. A number of rows appeared to be segregating in a ratio of about 3 resistant to 1 susceptible, and in a few rows the ratio of resistant to susceptible individuals exceeded 20:1. All F_3 rows from susceptible F_3 plants bred true for susceptibility except that several F_3 lines included a single resistant plant or rarely 2 resistant plants, which were considered to be natural hybrids.

The observed segregation in F_2 and F_3 appears to be best explained by linked duplicate genes. Eleven sets of data were available for estimating the recombination value under this hypothesis. These were: 8 F_2 populations, 2 sets of F_3 lines raised from unclassified F_2 individuals, and a single set of F_3 lines raised from stem rust resistant F_2 individuals. The recombination value, p, was estimated by the method of maximum likelihood (4). In deriving the maximum likelihood equation, the expectancy for the repulsion-phase double heterozygotes was included among the segregating genotypes. Because such F_2 genotypes produce few susceptible progeny in F_3, a susceptible plant might not always have occurred in F_3 progenies of the size grown. However, the error introduced by misclassifying a portion of such progenies as homozygous-resistant would be small because of the infrequency of the repulsion heterozygote with close linkage.

The logarithmic likelihood expression for the combined data is $L = 1556\text{–}(3+2p-p^2) + 457\text{–}(1-2p+p^2) + 196\text{–}(1+2p-p^2)$. Maximization with respect to p and solution for p gives a value of 0.1478. The variance of the recombination fraction was calculated from the equation

$$-\frac{1}{S^2p} = \frac{d^2L}{dp^2},$$

giving a value of 0.0003058 for S^2p. Thus, a recombination fraction of 14.78 ± 1.75 per cent is indicated.

A method described by Mather (4) was used to test the homogeneity of the combined F_2 and F_3 data. The heterogeneity χ^2 was obtained from the equation

$$\chi^2 = \Sigma \frac{D^2}{I}$$

where D is the deviation from expectation and I is the amount of information about p contributed by each body of data. I was calculated as the negative of the second derivative of the logarithmic likelihood expression. Results of this heterogeneity test are in Table 4. The small total deviation from zero indicates a good fit for p. The nonsignificant χ^2 indicates that the 11 sets of data are in agreement in supporting the hypothesis of duplicate factors, linked with a recombination value of 14.78 ± 1.75 per cent. In Tables 2 and 3, the observed frequencies may be compared with the numbers expected with 14.78 per cent recombination. There is good agreement with expectation in 10 of the 11 sets of data and a doubtful fit in only 1.

Subsequent to the calculation of the recombination value reported above, another F_2 population of each hybrid was classified for stem rust reaction at Madison. Results are in agreement with the hypothesis of duplicate linked genes (Table 6). Additionally, in an F_2 population of the hybrid Reward × C. I. 12633 grown at Davis in 1951, 489 resistant and 108 susceptible plants were observed, and among 358 F_3 families grown at the same time the ratio of homozygous resistant to segregating to homozygous susceptible families was 110:191:57. These results agree closely with those obtained in 1947, 1948, and 1950. The labor of reestimating the combination value to include these ad-

TABLE 3.—*Goodness of fit of F_3 segregation under the hypotheses that stem rust resistance is governed by a single dominant gene or by linked duplicate genes with a recombination value of 14.78 per cent*

Hybrid, place and year grown	Rust reaction	Obs.	1:2:1 or 1:2 ratio		Duplicate linked factors with 14.78 per cent recombination	
			Expected	Probability	Expected	Probability
Reward × C. I. 12632, Madison, 1948	Homozygous resistant	78	57	<0.01	72.60	0.27
	Segregating	118	114		114.00	
	Homozygous susceptible	32	57		41.40	
Reward × C. I. 12633, Madison, 1948	Homozygous resistant	91	65.75	<0.01	83.75	0.60
	Segregating	124	131.50		131.50	
	Homozygous susceptible	48	65.75		47.75	
Marquis × C. I. 12633,[a] Davis, 1948	Homozygous resistant	27	20	0.12	23.35	0.34
	Segregating	33	40		36.65	

[a] Only F_3 lines from resistant F_2 plants were grown.

TABLE 4.—*Agreement of eleven sets of data in support of the hypothesis that duplicated linked genes with a recombination value of 14.78 per cent govern stem rust resistance*

Hybrid and generation	Location	Year	Deviation (D)	Information (I)	$\frac{D^2}{I} = \chi^2$
F_2:—					
Reward × C. I. 12632	Madison	1947	+40.6948	364.7992	4.5397
Reward × C. I. 12632	Madison	1948	−11.3283	201.5747	0.6366
Reward × C. I. 12632	Madison	1950	+ 4.3888	68.6531	0.2806
Reward × C. I. 12633	Madison	1947	+ 3.3930	423.4406	0.0272
Reward × C. I. 12633	Madison	1948	−11.9045	294.6878	0.4809
Reward × C. I. 12633	Madison	1950	−26.4902	282.1236	2.4873
Reward × C. I. 12633	Davis	1947	−28.6457	399.1740	2.0557
Marquis × C. I. 12633	Davis	1947	−13.5475	426.2644	0.4306
F_3:—					
Reward × C. I. 12632	Madison	1948	+29.2714	310.2644	2.7616
Reward × C. I. 12633	Madison	1948	+ 9.1167	424.9679	0.1956
Marquis × C. I. 12633	Davis	1948	+ 4.8909	74.0649	0.3230
Total			− 0.1606	3270.0146	14.2188[a]

[a] $P = 0.17$ Homogeneity is indicated.

ditional data was not considered justified in view of the small alteration in the value of p which would result.

Critical information regarding the inheritance of stem rust resistance was obtained from a repeat planting in 1951 of an F_3 family which produced 51 resistant plants and a single susceptible plant in 1948, and was presumed to represent a repulsion-phase double heterozygote. In this family 438 resistant and 4 susceptible plants were observed in 1951, or a total of 489 resistant and 5 susceptible plants for the 1948 and 1951 seasons combined. Since 3 susceptible plants were expected under the duplicate linked gene hypothesis, the results are in accord with expectation.

Data obtained at both Madison and Davis in 4 different seasons are therefore consistent in indicating that dominant duplicate genes, separated by approximately 15 crossover units, govern stem rust resistance in these hybrids. It must also be concluded that 1 or both of these genes are distinct from the genes determining stem rust resistance in Timstein (9).

INHERITANCE OF RESISTANCE TO POWDERY MILDEW.—*Field tests.*—Natural epiphytotics of powdery mildew and leaf rust occurred at Madison in 1948 allowing F_3 families of the hybrids Reward × C. I. 12632 and Reward × C. I. 12633 to be classified simultaneously for reactions to mildew, leaf rust, and stem rust. Determination of adult plant reaction to mildew was made just after heading and to leaf rust and stem rust shortly before maturity. The average size of the F_3 families was 45 plants. In Reward an average of 27 per cent of the leaf area was covered with mycelium but this varied from 10 to 40 per cent which indicated that severity of the mildew was not uniform. A trace of mildew occurred on 1 of the 6 rows of C. I. 12632 and on 3 of the 8 rows of C. I. 12633.

The segregation for reaction to mildew was not so clear cut as the segregation for reaction to stem rust. Five types of F_3 lines were observed. These were:

Class 1. Resistant; plants uniformly with no mildew infection or only a trace; equal in resistance to C. I. 12632 or C. I. 12633.

Class 2. Moderately resistant; included families with 1, 2, or 3 per cent of the leaf area infected and families segregating from 0 to 3 per cent.

Class 3. Segregating; mildew coverage of the leaf area varied widely within families, such as 0–20, 0–50, or 5–50 per cent. Resistant plants predominated.

Class 4. Moderately susceptible; between-family infection ranged from 5 to 20 per cent of the leaf area covered with mycelium.

Class 5. Susceptible; families varied, 5–50 per cent of the leaf area being covered with a profuse growth of mycelium. The families approached the mildew reaction of Reward, but there appeared to be variability within and between families.

Frequencies of the several types of F_3 families are in Table 5. The 78 families from Reward × C. I. 12632 that were homozygous for resistance to stem rust were either resistant or moderately resistant to mildew with the exception of 3 that appeared to segregate widely for reaction to the latter disease. All lines homozygous for susceptibility to stem rust were susceptible or moderately susceptible to mildew. The correlation in response to the 2 diseases was even more definite in F_3 families from Reward × C. I. 12633. In that hybrid, all families homozygous for resistance to stem rust were either resistant or moderately resistant to mildew and all families homozygous for susceptibility to stem rust were either susceptible or moderately susceptible to mildew. These results suggest a close linkage between the 2 factors determining stem rust reaction and the factors determining adult plant response to powdery mildew. There appeared to be no relationship between reaction to leaf rust and reaction to mildew or stem rust.

THE RELATIONSHIP BETWEEN RESISTANCE TO MILDEW AND TO STEM RUST.—A series of greenhouse and field tests were conducted in order to gain more information on the type of association between stem rust and mildew. Seedling populations of 57 F_2 plants of Reward × C. I. 12632 and 214 F_2 plants of Reward × C. I. 12633 were subjected to a greenhouse test for mildew reaction in 1950. After classification for mildew reac-

TABLE 5.—*Joint segregation in the field for stem rust and powdery mildew adult reactions of F_3 families from crosses of Reward with C. I. 12632, and C. I. 12633, Madison, 1948*

Cross, parents, and F_3 stem-rust reaction	Number of F_3 families or rows in indicated mildew classes[a]					
	1	2	3	4	5	Total
Reward × C. I. 12632						
Homozygous resistant	24	51	3	0	0	78
Segregating	1	27	83	7	0	118
Homozygous susceptible	0	0	0	20	12	32
Total	25	78	86	27	12	228
Parents						
Reward				2	5	
C. I. 12632	6	1				
Reward × C. I. 12633						
Homozygous resistant	18	73	0	0	0	91
Segregating	0	16	108	0	0	124
Homozygous susceptible	0	0	0	31	17	48
Total	18	89	108	31	17	263
Parents						
Reward				1	7	
C. I. 12633	5	3				

[a] See text for explanation of classes.

tion, the seedlings were transplanted to the field. F_2 adult reaction to stem rust was obtained, seeds were harvested at maturity, and F_3 families were tested in the greenhouse for both seedling and adult responses to mildew in 1951. In addition, a few F_4 families were tested in the greenhouse for both seedling and adult reactions to mildew in 1952. These tests made it possible to study the interrelationships between seedling mildew reaction, adult mildew reaction, and adult stem rust reaction.

The inoculum employed in greenhouse tests was race 5 of *Erysiphe graminis tritici* as determined by M. C. Futrell with varieties listed by Newton and Cherewick (6). Seedlings were inoculated with conidia shortly after emergence, and seedling reaction to the disease was recorded for the blade of the first seedling leaf. When adult plant reaction also was to be determined, the plants were inoculated with conidia 2 more times as additional leaves developed, and the reading was made when the plants had 5 or 6 leaves. The second leaf of plants moderately resistant as adults was considered to be transitional since a proximal portion of this leaf usually gave a seedling response and a distal portion an adult response. Greenhouse temperatures were maintained at 16°–18° C. during these tests.

Seedling mildew and adult stem rust reactions.—The reaction of F_1 plants to mildew was the same as that of the resistant parents in the seedling stage. F_2 plants fell into 3 classes—resistant, intermediate, and susceptible. Resistant plants were free from or had traces of mildew, but some of the plants showed a flecking of the leaf. The intermediate response was characterized by a sparse growth of mycelium, and the susceptible response by profuse growth of mycelium on the blade. After classification, these plants were transplanted to the field and their stem rust was determined. The relationship between seedling reaction to mildew and reaction to stem rust for the 2 crosses is in Table 6. Since a number of the plants that were resistant to mildew in the seedling stage were susceptible to stem rust, and the converse was also true, it is apparent that the close association between adult stage responses to the 2 diseases does not exist in the present comparison.

Comparison between seedling and adult responses to mildew.—The relationship between seedling and adult responses to mildew was tested in 2 ways. The first method involved a comparison between the mildew reactions of F_2 seedlings, determined in 1950, and the adult reactions of F_3 families derived from these seedlings. It is clear from the data in Table 7 that the agreement in response at the 2 stages was not close. In the second method, (data not included) a comparison was made between the responses of the first leaf of F_3 plants and later leaves developed on the same plants. Plants whose seedling leaf blades had as high as 60 per cent of the area covered with mycelium sometimes fell in the 3 per cent class as adults. Again the evidence indicated that seedling and adult plant responses are under different control genetically.

F_2 stem rust and F_3 adult plant mildew responses.—The joint segregation for F_2 adult plant response to stem rust in the field and the adult plant mildew response in the greenhouse of F_3 families derived from the F_2 plants is in Table 8. In both crosses, all stem rust resistant F_2 plants produced F_3 progenies which were either moderately resistant to mildew as adults or were segregating for mildew reaction. F_2 plants susceptible to stem rust produced F_3 progenies that were either moderately susceptible or susceptible to mildew as adults. The F_3 families were carefully examined for possible crossovers between the 2 genes governing reaction to stem rust and the genes governing mildew reaction. Twelve of the F_3 families from the hybrid Reward × C. I. 12633 were selected for retesting because their reaction to one or the other disease was not definite. A field rust test clarified the

TABLE 6.—*Relationship between the F_2 seedling reaction to mildew in the greenhouse and the adult plant reaction to stem rust in the field of the same F_2 plants from crosses of Reward with C. I. 12632 and C. I. 12633. Madison, 1950*

Cross and F_2 adult stem rust reaction	Number of F_2 seedlings in mildew classes indicated[a]			
	Resistant	Intermediate	Susceptible	Total
Reward × C. I. 12632				
Resistant	28	15	5	48
Susceptible	1	4	4	9
Total	29	19	9	57
Reward × C. I. 12633				
Resistant	90	34	42	166
Susceptible	2	33	13	48
Total	92	67	55	214

[a] See text for explanation of classes.

TABLE 7.—*Relationship between the F_2 seedling reaction in 1950 and the F_3 family adult reaction to mildew in 1951 in greenhouse tests from crosses of Reward with C. I. 12632 and C. I. 12633. Madison*

Cross and mildew reaction of F_2 seedlings	Number of adult F_3 families in mildew classes indicated[a] 1	2	3	4	5	Total
Reward × C. I. 12632						
Resistant	1	14	13	0	1	29
Intermediate	0	8	7	2	2	19
Susceptible	0	0	5	0	4	9
Total	1	22	25	2	7	57
Reward × C. I. 12633						
Resistant	0	42	48	1	1	92
Intermediate	0	10	29	0	28	67
Susceptible	0	11	29	1	14	55
Total	0	63	106	2	43	214

[a] See text for explanation of classes.

status of 6 of these families, making it possible to assign them to their correct place in Table 8. The indefinite status of the remaining families, which were susceptible to rust, was associated with their reaction to mildew, necessitating additional greenhouse tests.

RELATION OF NECROTIC RESPONSE TO MILDEW REACTION.—F_4 families derived from the 6 F_3 families with ambiguous reaction to mildew were inoculated in the greenhouse with conidia of race 5 shortly after emergence and again at a later date. Each F_4 line was represented by approximately 15 seedlings. Careful inspection of the seedlings revealed 2 different responses to mildew, necrotic and non-necrotic. With the necrotic response, considerable areas of the first leaf blade became chlorotic. Such areas were dead by the eighth day after inoculation. The necrotic areas increased in size until most of the first blade of many plants was dead 25 days after the first inoculation. The necrotic response did not occur on Reward. After heavy inoculations with conidia, C. I. 12633 developed small necrotic spots that coalesced on the first blade but tended to cause flecking on the higher leaves. C. I. 12633 and some F_4 plants showed necrosis on the leaf sheath also. In general, the necrosis developed faster and became more prominent on F_4 plants than on C. I. 12633 in this test, which was terminated with the fourth leaf.

A significant feature of the necrotic response was a reduction in the amount of mycelium on the first 4 leaf blades in comparison with similar blades of plants not showing the necrotic response. On the second, third, and fourth leaf blades of plants showing necrosis there was less necrotic area and more mycelial growth than on the first blade. Occasional third and fourth blades showed flecking. The area infected with mildew averaged 12.4 per cent for the first 4 blades of those plants showing the necrotic response compared to 47.7 per cent for segregates without necrosis.

Of the 6 F_3 families represented in the test, 4 were segregating and 1 was homozygous for the leaf blade necrosis. The sixth family was probably segregating

TABLE 8.—*Joint segregation for adult F_2 plant reaction to stem rust in the field in 1950 and F_3 family adult reaction to powdery mildew in the greenhouse in 1951 from crosses of Reward with C. I. 12632 and C. I. 12633. Madison*

Cross and F_2 stem rust reaction	Number of F_3 families or parents in indicated mildew classes[a] 1	2	3	4	5	Total
Reward × C. I. 12632						
Resistant	0	23	25	0	0	48
Susceptible	0	0	0	2	7	9
Total	0	23	25	2	7	57
Parents						
Reward					3	
C. I. 12632	2	1				
Reward × C. I. 12633						
Resistant	0	64	102	0	0	166
Susceptible	0	0	0	3	45	48
Total	0	64	102	3	45	214
Parents						
Reward					7	
C. I. 12633	1	5				

[a] See text for explanation of classes.

but it could not be classified with certainty because it was represented by a single F_4 line. Plant counts in the 11 segregating F_4 lines showed a ratio of 3 necrotic to 1 non-necrotic plants. The necrotic response is apparently governed by a single dominant Mendelian factor carried by C. I. 12632 and C. I. 12633. It is clear that this gene imparts partial resistance to mildew and is responsible for the intermediate reaction to mildew of the 6 families discussed above. Apparently these 6 families did not carry the adult mildew resistance associated with the stem rust resistance genes. This conclusion is consistent with their susceptibility to stem rust.

The necrotic response was also observed in certain F_3 families that were segregating for the adult mildew resistance associated with stem rust resistance. The manner in which the necrotic gene affected the mildew response of segregates with adult plant resistance was not determined. It is possible that plants with both adult mildew resistance and the necrotic response fall into Class 1 (highly resistant), whereas the adult plant resistance factors associated with stem rust resistance produce moderate resistance to mildew (Class 2) in the absence of the necrotic factor.

When necrotic and non-necrotic plants were compared in late April at higher temperatures, small differences in the area covered with mycelium were observed. Apparently this gene is more subject to environmental influences than the mildew resistance genes associated with stem rust resistance.

DISCUSSION.—The evidence obtained from field trials in several seasons at each of 2 locations indicates that the stem rust resistance of C. I. 12632 and C. I. 12633 is governed by duplicate genes located about 15 crossover units apart. The existence of 2 such units in the same chromosome may be the result of duplication of

a small segment. Nonlinked duplicate genes have been described repeatedly, and a few cases of very closely linked "tandem repeats" are known (see 12 for discussion). If the 2 loci in question represent a duplication, they differ from previously described cases mainly in the tightness of the linkage. While the duplicated unit is situated in the same chromosome, its location is relatively remote contrasted to "tandem repeats."

The evidence was also clear that a very close association exists between the 2 genes governing stem rust resistance and the gene or genes governing adult plant resistance to powdery mildew. This type of resistance did not express itself appreciably on the blade of the first leaf or the distal half of the blade of the second leaf, but it prevented infection by mildew of more than 3–5 per cent of the leaf blade area on the third or higher foliar leaves of the plant. Another type of mildew resistance was governed by a single factor pair, the dominant allele causing a necrotic reaction on the lower leaves of the plant and a reduction in the prevalence of mycelial growth on both seedlings and adults. These 2 types of resistance do not explain all the heritable differences that appeared in the 2 crosses studied. Undoubtedly 1 or more other genes modifying seedling or adult responses were involved, but the number or interactions of these additional genes for mildew resistance could not be determined.

Probably the question of greatest interest in these hybrids is the exact relationship between stem rust resistance and adult plant mildew resistance. Complete association was found between stem rust reaction in the field of 271 F_2 plants and the adult plant mildew reaction in the greenhouse of F_3 families derived from these plants. The association between rust resistance and adult plant mildew resistance was not so clear-cut in a field trial conducted in 1948, because a few F_3 families among the 491 classified appeared to be homozygous for resistance to one disease and segregating for reaction to the other. Among the 124 lines segregating for stem rust reaction in the hybrid Reward × C. I. 12633, 16 appeared to be homozygous for resistance to mildew; in the hybrid Reward × C. I. 12632, 118 lines segregating for stem rust reaction included 28 which appeared to be homozygous for mildew resistance. However, some classifications for reaction to mildew in this trial may have been inaccurate. The classification was made shortly after heading and possibly before the mildew epiphytotic had developed fully. Also the epiphytotic was not uniform throughout the nursery. Under such conditions, together with the genetic variability caused by the segregation of the necrosis factor and possibly other genes, it may be that some lines heterozygous for adult plant mildew resistance genes were classified as homozygous for resistance.

Among the 78 lines of the hybrid Reward × C. I. 12632 which were homozygous for stem rust resistance, 3 appeared to be segregating widely for reaction to mildew, and among 118 lines segregating for stem rust reaction 7 apparently were susceptible to mildew. In contrast to the lines discussed above, there is greater reason to believe that these lines were crossover types because reasonable confidence was felt in the classification for stem rust reaction. However, classification for disease reaction is rarely absolute, so that the data do not establish with certainty the occurrence of any crossovers between the 2 linked genes governing stem rust resistance and the genes governing adult mildew response.

In the absence of more precise data, the observed segregation seems equally capable of explanation by any 1 of 3 Mendelian schemes. It is possible that several genes for mildew resistance are situated within the chromosome segment in question. This interpretation cannot be excluded because adult plant mildew resistance could not be associated precisely with only 2 Mendelian units as was possible with resistance to stem rust.

The segregation observed can be explained equally well by postulating 2 genes for adult mildew response, 1 such gene being very closely linked with each of the genes governing stem rust reaction. This interpretation implies that each of the 2 loci is "compound," consisting of a gene for mildew resistance and a gene for stem rust resistance. The existence of 2 "compound" units of this sort is most easily explained by duplication. In fact, the plausibility of such an origin may be an argument favoring the hypothesis of duplicate "compound" loci.

The third interpretation is that resistance to the 2 diseases results from dual effects of the same Mendelian units. It implies that each locus is simple, including only a single gene which controls the reaction or reactions leading to resistance to both stem rust and mildew. Mechanically, this and the previous scheme are virtually identical. As to method of gene action, however, their implications are quite different.

Close linkages between genes governing disease resistance in wheat have been reported previously by 2 investigators. In the hybrid between Federation and Thew, Waterhouse (13) found resistance to leaf rust and mildew to be tightly linked. He stated that further extensive tests would be required to determine whether or not this linkage can be broken. Neatby (5) found that F_6 lines from the cross H44-24 × Marquis, which were resistant in the seedling stage to race 36 of stem rust, were invariably susceptible to mildew and, conversely, all mildew resistant lines were susceptible to stem rust.

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PHYTOPATHOLOGICAL NOTES

Bacterial Soft Rot on Tomato Fruits affected by the Late Blight Fungus. Z. Volcani and I. Wahl.[1] Tomato fields in Israel were surveyed frequently in late April, 1951. Rapid development of soft rot was observed in tomato fruits that had been severely affected with late blight, after a change from cool to warm weather.

The fungus, *Phytophthora infestans* (Mont.) DBy. and a bacterial organism were isolated from the necrotic tissues; only the bacteria were isolated from the soft tissues bordering the necrotic ones. The cultural and physiological characteristics of the bacterium were those of *Bacillus subtilis* Cohn emend Prazmowski, which in Israel causes diseases of fruits of *Capsicum annuum* L. and *Solanum melongena* L.[2]

An association between bacterial rots and late blight in potato tubers was mentioned by several workers. Delacroix[3] in his observations of dry and wet rot of potatoes, noted also intermediate types of rot in which both fungi and bacteria were associated with *P. infestans*. Murphy and McKay[4] commented upon the 2 main types of rot. More recently Wilson and Boyd[5]

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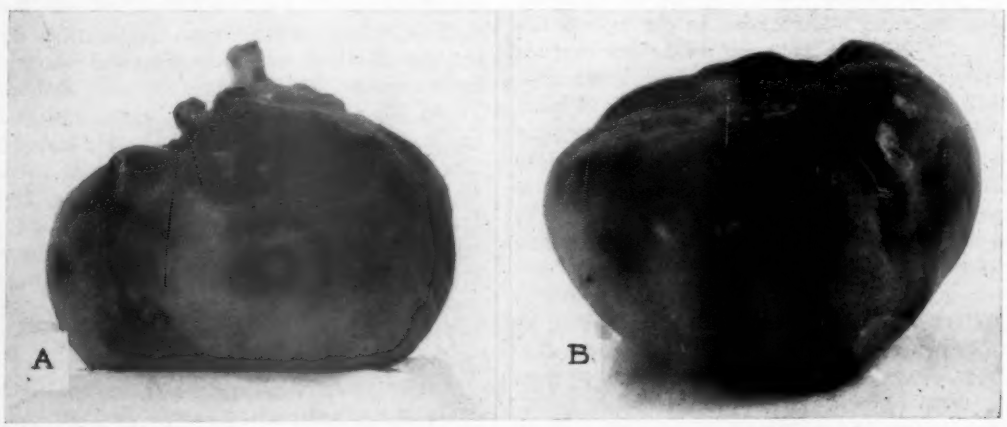

Fig. 1. A) Late blight and soft rot in green tomato fruits, resulting from inoculation with *Phytophthora infestans* at 20°C., followed by inoculation with *Bacillus subtilis* and incubation at 33°C. B) Late blight resulting from inoculation with *P. infestans* at 20°C., followed by inoculation of *Bacillus subtilis* and incubation at 20°C.

stressed the fact that wet rot is invariably associated with *P. infestans*. Dowson and Rudd Jones[6] have proved that this fungus produces favourable conditions for growth of bacteria, and that the saprophytic species of bacteria tested did not move beyond the area of the tuber invaded by *P. infestans*.

In preliminary infection experiments, soft rot was readily induced in disease-free tomato fruits by depositing a concentrated bacterial suspension on epidermal slits made with a sterile needle and then incubating the fruits under high relative humidity at 30-33°C. for 3 or 4 days. No infection occurred if inoculated fruits were incubated at 20-23°C. Furthermore, inoculations on uninjured fruits failed at either the high or low temperatures.

Further experiments were completed to investigate the relation of late blight infection to soft rot. In all tests, the fruits were kept under high relative humidity. Healthy, uninjured, green tomato fruits of uniform size were inoculated with *P. infestans* by placing small pieces of a fungus culture around the stem end of the fruit; they were then incubated at 20°C. for 6–7 days. The blighted fruits then were smeared with a concentrated bacterial suspension and divided into 2 series. One series was incubated at 33°C. and the second at 20°C. Within 2–3 days all fruits in the series at 33°C. were soft and rotten (Fig. 1A), but no soft rot developed in the fruits at 20°C. during that time or later. (Fig. 1B).

In other experiments, inocula of the fungus and of the bacterium were placed simultaneously around the stem ends of healthy, uninjured, green tomato fruits. Again the fruits were divided into 2 series, incubated at 20 and 33°C. respectively. After 6–7 days, late blight appeared in the fruits incubated at 20°C. but not in those incubated at 33°C. The higher temperature was unfavorable for the growth of *P. infestans*. No soft rot developed on any of the fruits at either temperature. Nevertheless, the fruits that were blighted after incubation at 20° C. were again divided into 2 lots. The first lot was further incubated at 33°C., while the second lot remained at 20°C. Soft rot developed 3 or 4 days later on the blighted fruits transferred to 33°C., whereas it failed to develop on those that remained at 20°C. Bacteria re-isolated from the soft rot appeared to be identical with the original isolates. The experiments were repeated with the re-isolated bacteria and results were the same.

It is concluded that *B. subtilis* may induce soft rot in injured tomato fruits if temperatures are relatively high and that late blight infection in tomato fruits makes possible the ingress of the bacteria. Furthermore, once the bacteria invade the tissue, they spread far beyond the necrotic area affected by *P. infestans;* this observation contrasts with that of Dowson and Rudd Jones[6] who reported that the bacteria were limited to the blighted area. Since high temperatures are inimical to the late blight fungus, neither late blight nor soft rot will develop in uninjured fruits at high temperatures, despite the fact that high temperatures favor the soft rot bacteria.—Agricultural Research Station, Rehovoth, and The Hebrew University, Jerusalem, Israel.

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Caryophyllaceae Susceptible to the Carnation Wilt Fusarium.[1] Joanne K. Armstrong and G. M. Armstrong.[2] A *Fusarium* wilt of sweet william (*Dianthus barbatus* L.) was reported by Snyder[3] and described as a new form (*Fusarium oxysporum* f. *barbati*) since it did not attack carnation (*Dianthus caryophyllus* L.). The varieties of carnation in these tests were not given, and sweet william was not inoculated with the carnation wilt *Fusarium* [*F. dianthi* Prill. & Del.[4] or *F. oxysporum* Schlecht. *f. dianthi* (Prill & Del.) Snyd. & Han.[5]]. Since some varieties of carnation possess wilt resistance,[6,7] and certain wilt *Fusaria* attack more than one host, the status of the new form on sweet william is uncertain. A study of the host ranges of the wilt *Fusaria*, having as the ultimate goal a revision of the section Elegans, has been undertaken by the authors; therefore, it was evident that an investigation of the host relationships of the carnation wilt *Fusarium* should be made. The results of this study are presented.

Experimental methods.—The methods of growing the plants in steamed sand and inoculating them with a liquid inoculum have been described.[8] The medium for the inoculum was as follows: sucrose or dextrose 2 per cent; $MgSO_4$, 0.003M; KCl, 0.022M; KH_2PO_4,

[1] Contribution of the Department of Botany and Bacteriolgy in cooperation with the Section of Cotton and Other Fiber Crops and Diseases, Field Crops Research Branch, U. S. Department of Agriculture. Technical contribution No. 216, South Carolina Agricultural Experiment Station.

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[2] Agent, Section of Cotton and Other Fiber Crops and Diseases, and Head, Department of Botany and Bacteriology, respectively.

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TABLE 1.—*Susceptibility of some Caryophyllaceae to the carnation wilt Fusarium*

Host	Number of plants with wilt symptoms: External	Internal only	None	Host	External	Internal only	None
D. caryophyllus:				*D. deltoides*	9	0	4
Apollo	13[a]	0	0	*D. plumarius*	9	1	0
Orion	35	0	0	*D. chinensis:*			
Jupiter	34	0	0	Heddewigii laciniatus, Snowball	118[c]	10	7
D. barbatus:				Heddewigii, The Bride	18	2	1
Newport Pink	77	0	0	Laciniatus splendens	21	4	0
Giant Pure White	387[b]	11	25	Westwood Beauty	48	1	1
Scarlet Beauty	31	0	0	*Gypsophila paniculata*	0	13	20
Copper Red	28	0	0	*Cerastium tomentosum*	0	0	40
Crimson Beauty	41	2	0	*Lychnis chalcedonica*	49	0	0
D. allwoodii alpinus	25	2	0				
D. caesius	12	1	2				

[a] Data for 1 isolate.
[b,c] Data for 7 and 4 isolates, respectively; other data for 2 isolates.

0.008M[9]; Ca $(NO_3)_2$, 0.0356M[9]; $FeCl_3$, $MnSO_4$, and $ZnSO_4$, 0.2 p.p.m. each of cations. Five to 7 rooted cuttings of carnation were planted per pot and, when well established, were inoculated with the carnation wilt *Fusarium* by cutting the roots and pouring the inoculum around them. Seed of the other Caryophyllaceae were sown in pots. The plants were thinned to 10-15 per pot and, when 4-6 weeks old and about 2 in. high, were inoculated in the same manner as the carnations. All the fungus isolates were derived from single spores. Noninoculated plants of each species or variety grown concurrently with the inoculated ones showed no external or internal symptoms of wilt. The investigations extended over a period of 2 years, during the fall, winter, and spring when the temperature in the greenhouse could be kept at approximately 28°C.

DISCUSSION AND RESULTS.—An isolate of the carnation wilt *Fusarium* was used to inoculate plants of Grimm alfalfa, Mexican Red bean, Copenhagen Market cabbage, *Cassia tora* L., Golden Self-blanching celery, Hurley's Rowden cotton (*Gossypium hirsutum* L.), Sakel cotton (*G. barbadense* L.), Kumpta cotton (*G. herbaceum* L.), California Blackeye 5 and Lady Finger cowpea, dill, mimosa (*Albizzia julibrissin* Duraz.), Hale's Best muskmelon, Thomas Laxton pea, Long White Icicle radish, Criollo sesame, Yelredo soybean, Bloomsdale Savoy, Nobel and Aragon spinach, Ten-weeks Bright Pink stock, G. W. 359 sugar beet, sumac (*Rhus typhina* L.), Porto Rico sweet potato, Ky. White Burley and Gold Dollar tobacco, Bonny Best tomato, annual single mixed wallflower, and Tom Watson watermelon. One pot of plants of each variety was used in the inoculations with 5 or 6 plants per pot of tobacco, tomato, watermelon, and sweet potato, and 10 or more for the others. No external or internal symptoms of wilt appeared in any plant.

Giant Pure White sweet william and Apollo carnation were inoculated with wilt *Fusaria* from the following hosts: alfalfa; bean; cabbage and radish (races 1 and 2); Cassia; cotton from India, Egypt, and the U.S.A.; cowpea (races 1 and 2); mimosa (race 1); muskmelon; English pea (race 2); sesame; spinach; stock; sugar beet; sumac; sweet potato; flue-cured tobacco; tomato (race 1); and watermelon. No external or internal symptoms of wilt appeared in any plant.

Results of the preceding inoculations failed to indicate a relationship between the carnation wilt *Fusarium* and any of the 22 other *Fusaria* that were tested since a common host was not found.

All plant of the Caryophyllaceae, except Apollo carnation where 1 isolate was used, were inoculated with each of 2 carnation wilt isolates. In addition, 2 other isolates were tested separately on a variety of garden pink and sweet william only. Since no differences were noted in the pathogenicity of the cultures, the data for each of the Caryophyllaceae are combined in Table 1. Six species of *Dianthus*, in addition to *D. caryophyllus*, with several varieties of 2 of these, and *Lychnis chalcedonica* L. were highly susceptible; *Gypsophila paniculata* L. was highly resistant with only internal symptoms; *Cerastium tomentosum* L. showed no symptoms whatever.

An isolate of the sweet william wilt *Fusarium* was not available for comparison in the present investigation, but it is evident from Table 1 that it is either the carnation wilt *Fusarium* or a race of this organism with *D. barbatus* as the common host.

A common host was not found for the carnation wilt *Fusarium* and any of 22 other wilt *Fusaria*.—South Carolina Agricultural Experiment Station, Clemson, South Carolina, and U. S. Department of Agriculture.

[9] Misprint in Phytopathology 38: 809. 1948. where these salts were 0.1008M and 0.10356M, respectively.